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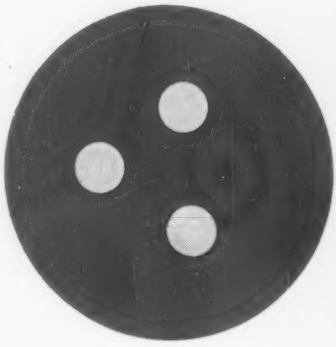

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HENRY G. MAUTNER*

Department of Pharmacology, Yale University School of Medicine

PROBLEMS IN THE DESIGNING OF ANTIMETABOLITES†

Historically, the most useful medicinal agents have been obtained by empirical screening or by the chemical modification of compounds of known activity in the hope of making them more effective. To these approaches a new one was added in recent years—antimetabolites.

An antimetabolite may be defined as a compound bearing structural resemblance to a metabolite and capable of being attached to the enzyme for which the metabolite serves as the normal substrate. The enzyme-antimetabolite complex, however, is incapable of undergoing the reactions of the enzyme-metabolite complex and, by interfering with the modification of the metabolite, a specific biochemical lesion is created.

The best example of truly effective antimetabolites is the sulfonamides, still among our most useful antibacterial agents. Sulfonamides are antimetabolites of p-aminobenzoic acid, which in turn is used by susceptible bacteria for the synthesis of coenzymes structurally related to pteroylglutamic (folic) acid.[20] The animal host cannot synthesize its own folic acid; most bacteria, on the other hand, cannot utilize preformed folic acid. Here, a clean-cut, qualitative metabolic difference between invading cells and the cells of the host has been exploited with considerable success.

After the discovery of the mode of action of the sulfa drugs which, incidentally, were found through screening synthetic dyes, rather than by the application of the principles discussed above, it seemed likely that the exploitation of biochemical differences between host and invading cells (whether bacteria, viruses, or neoplasms) would lead to the synthesis of a large number of potent drugs.

This approach, the design of antimetabolites, did lead to the synthesis of large numbers of organic compounds. However, while thousands of potential antimetabolites have been synthesized, the number of useful drugs among them has been disappointingly small. Certainly the most important medicinal agents to be introduced during the last twenty years, the antibiotics,

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* Assistant Professor of Pharmacology.

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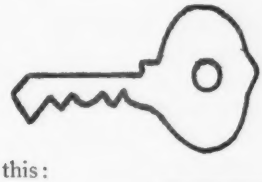
did not originate in chemical laboratories but in such unlikely places as, for instance, the wounded knee of a little girl, a rotten cantaloupe, or in some of almost innumerable samples of soil. These antibiotic compounds, which have revolutionized the treatment of bacterial diseases, are the fruit of purely empirical screening, even though their therapeutic usefulness has in some cases been improved by chemical modification.

One of the reasons for the failure of so many antimetabolites may lie in the necessarily mechanical way in which they are being designed. Thus, as soon as the structure of a new metabolite is established, even when its biochemical role may not have been elucidated, dozens of potential "antimetabolites" are described in the literature within a matter of months. Generally, these are designed by the application of rather simple rules, for instance:

1. Only one substituent group should be changed at one time; multiple alterations in the structure of a metabolite are to be avoided. This rule is followed fairly generally, even though Amethopterin ("Methotrexate"), one of the most useful antileukemic agents now available, is a doubly modified analog of folic acid.[23]
2. Hydroxy (–OH) groups may be interchanged with amino ($-NH_2$) groups. Examples: aminopterin[21]; 2,6-diaminopurine.[5]
3. Oxygen may be interchanged with sulfur or selenium. Examples: 6-mercaptopurine,[11] 6-selenopurine.[22, 28]
4. Carboxyl (–COOH) groups may be interchanged with sulfonamide ($-SO_2NH_2$) or methylsulfonyl ($-SO_2CH_3$) groups. Examples: the sulfonamides, uracil-6-methylsulfone.[14]
5. Methyl ($-CH_3$) groups may be replaced by bromine or iodine. Examples: 5-bromouracil,[18] 5-iododeoxyuridine.[20]
6. Hydrogen may be replaced by fluorine. Examples: fluroacetate,[26] fluorocitrate,[26] 5-fluorouracil.[5]
7. In heterocyclic compounds, carbon and nitrogen may be exchanged in the ring. Example: 8-azaguanine,[24] pyrazolo-[3,4-d] pyrimidines,[21] 6-azauridine.[19]
8. Alkylating groups (for instance the $-CHN_2$ or the $-N(CH_2CH_2Cl)_2$ group) may be introduced. Example: azaserine,[4] 5-bis(β-chloroethyl) aminouracil.[21]

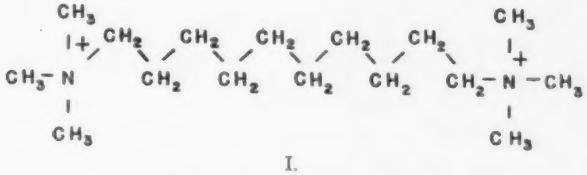
Unfortunately, for antimetabolites to be effective one needs not only a list of the metabolite's replaceable groups, but also some information about the functions of these groups and concerning the nature of the receptor sites to which they are to be attached. Otherwise, the chemotherapist is in the

position of a man who, on being asked to jam a lock by altering a key looking like this,

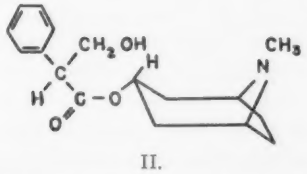


changes it to look like this:

since he did not know which end of the key was functional. This analogy also suggests that metabolites and antimetabolites do not—as stated by Woolley—have to resemble each other as much as possible, but that their structure has to be similar primarily in that part of the molecule which fits an enzyme surface. Thus, decamethonium (I) and atropine (II), which certainly show

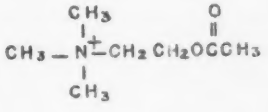


I.



II.

little structural resemblance to acetylcholine (III), can attach themselves to some of its receptor sites. Decamethonium can mimic the effects of acetylcholine at the neuromuscular junctions[19];

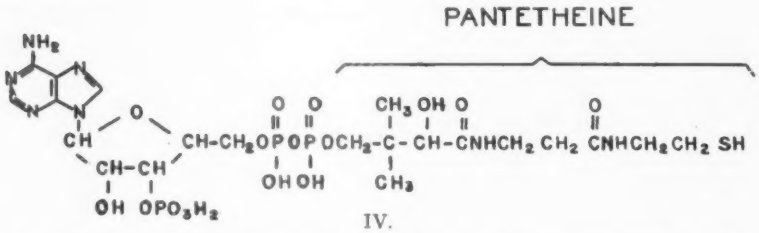


III.

atropine is a competitive antagonist of acetylcholine at post-ganglionic parasympathetic endings.[10] It can be seen that through the use of such considerations the term antimetabolite could be extended to include a large number of existing medicinal agents.

Unfortunately, information about substrate-enzyme interaction in terms of chemical mechanisms is very scarce and in most cases antimetabolites have to be designed without knowing which groups of the metabolite are functional. Even when a group known to be essential can be replaced by another, there can be no certainty that the change made will not be too great, in which case structural specificity is lost, or too small, in which case the analog may even be as functional as the compound which it mimics.

An example of a change of the latter type can be seen in selenopantethine,[18] the selenium analog of the sulfur compound, pantethine, which is the major component of coenzyme A(IV). The metabolic functions of this coenzyme are exerted through the formation of S-acyl intermediates,[20] so that the chemical reactions of the whole complex molecule can be referred to its single atom



IV.

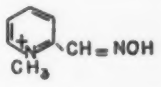
of sulfur. Yet, when this sulfur was replaced by selenium, it was found that a microorganism requiring preformed pantethine grew just as well, on a mole for mole basis, on its selenium analog.[20] Although it remains to be established that the organism, grown on the seleno-compound, contains a biologically functional seleno-coenzyme A, the probability of this is great.

Another difficulty in working with antimetabolites relates to the assumption that drug and metabolite will behave differently only at the receptor site where inhibition is to take place, at which point antagonism will occur. At the same time, the tacit assumption is made that everywhere else in the body, in terms of metabolism, ability to be carried to the site of action, and toxicity unrelated to the site of action, metabolite and antimetabolite will behave in an identical fashion. This assumption, as would be expected, is hardly ever valid, since the replacement of chemical groups on a substrate by other groups usually alters the dissociation constant, lipid solubility, chemical reactivity, and paths of metabolic alteration of the molecule.

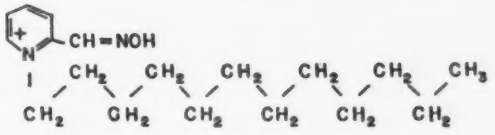
Changes in acid dissociation constant are particularly important, since the degree to which a drug is dissociated at physiological pH determines to what extent attachment to, or passage past, charged groups in biological tissues may occur. Since degree of dissociation stands in an inverse relationship to lipid solubility, it can be seen that as the pK_a of a compound is altered, its ability to reach its site of action will be altered too.[1] This is important, since anything that can be done to enable a functionally effective antimetabolite to reach its site on the biophase more efficiently than the metabolite will almost certainly make it a better antagonist.

Lipid solubility, as well as the degree of dissociation, is also important in enabling drugs to cross the blood-brain barrier.[6,7] Failure to cross this "barrier" may increase the probability of resistant cells emerging within the brain[85] and greatly diminishes the value of drugs used in the treatment of diseases in which the central nervous system may be invaded by microorganisms or malignant cells.

If the fat solubility of a drug is too low, there are several methods by means of which it may be increased. The most widely used procedure is the introduction of long alkyl chains. This usually has the desired effect; however, the introduction of bulky groups changes the ability of drugs to attach themselves to receptor sites and may alter their mechanism of action completely. In a recent example of this it was found that pyridine-2-aldoxime methiodide[87] (V), a reversing agent for acetylcholine esterase profoundly inhibited by "nerve gases," could not exert its effects in brain tissue, since the fat solubility of the aldoxime was too low. When its structure was modified in such a way that the methyl group of the aldoxime-derivative was replaced by the long-chain, lipid-solubilizing dodecyl group (VI), the new



V.



VI.

compound was very much more lipid soluble and able to affect structures uninfluenced by the parent compound. However, the introduction of the long alkyl chain gave the new compound depolarizing properties (which in

view of its similarity to decamethonium (I) is not too surprising) and increased its toxicity.[27]

Lipid solubility may also be increased by masking ionic groups, for instance by esterifying carboxyl or alkylating amino or hydroxy groups. However, since attachment to receptors is usually carried out by the groups now being altered, this procedure may result in loss of the desired activity.

It can be seen that in altering the fat solubility of a compound it is desirable to change its size, shape, and dissociation constants as little as possible. In some cases, this can be accomplished by replacement of oxygen by sulfur or even by selenium. In a recent study of several series of homologous heterocyclic oxygen, sulfur, and selenium compounds, it was found that this method of solubilization, which changes the configuration of the molecule very little, seems to be a generally effective one.[25]

The most important difficulty in designing antimetabolites, however, is not a chemical but a biochemical one. This is the frequent lack of qualitative metabolic differences between the cells of the host and the cells to be destroyed. This problem is particularly crucial in the field of cancer chemotherapy. Until now no enzymes have been found in malignant cells that are not present in normal cells.[15] While it has been reported recently that neoplasms may be profoundly deficient in some enzymes present in the host,[20] this finding would seem to be more useful for designing antimetabolites for freeing the tumor from the host than for doing the opposite! At best, it can be hoped that lack of catabolic enzymes may reduce preferentially the inactivation of a drug by tumor tissue.

Because of this lack of qualitative biochemical differences between normal and neoplastic cells, most carcinostatic antimetabolites are toxic to the cells of the host as well as to the cells of the tumors. Differential toxicity apparently is determined primarily by the greater rate at which the metabolite is depleted in the rapidly proliferating cells of the tumor as compared to the more slowly growing cells of the patient. This means that the therapeutic indices of these drugs tend to be quite low. When even a slight degree of drug resistance emerges, the dosage often cannot be raised enough to overcome it without causing intolerable toxicity to the host.

Since in designing antimetabolites it is important to decide just what one expects these compounds to do, it is convenient to subdivide them into several classes. These are:

1. *Reversible antimetabolites.* Here metabolite and antimetabolite compete for the same receptor sites. Since the inhibitor is bound comparatively loosely, it may be dislodged as the concentration of the metabolite is increased. The ratio of the two compounds at which inhibition takes place

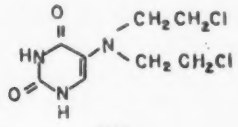
is a function of the relative firmness with which molecules of drug and molecules of normal substrate are held by the receptor once equilibrium has been established. If the concentration of both compounds is altered to the same extent, the degree of inhibition remains the same. The sulfonamides are representatives of the few effective drugs in this class.

2. *Irreversible antimetabolites.* Compounds which are bound so firmly that the metabolite cannot dislodge them at any concentration, may be subdivided into two groups:

a. *Endotoxic antimetabolites.* These compounds are similar to the reversible antimetabolites discussed above in that those groups of the metabolite are modified through which attachment to the receptor takes place; however, with endotoxic antimetabolites very firm binding will result. For example, it has recently been shown that aminopterin and amethopterin are bound much more firmly to folic acid reductase than is the normal substrate, folic acid.[86] Presumably the amino group of the inhibitor is held more strongly by an acidic site than the analogous hydroxy group of the metabolite.[2] It would seem likely that some essentially irreversible inhibitors of this type might become reversible as the pH of the system is changed.

b. *Toxophoric antimetabolites.* This name is proposed for antimetabolites carrying a highly reactive group (bis(β-chloroethyl)amino-, or diazomethyl groups, for instance) unrelated in structure and reactivity to any group of the normal metabolite. Since such substituents are rather bulky as well as reactive, it would seem desirable that they should not replace any groups responsible for attachment of the metabolite to the enzyme. Such replacement would be expected to result in loss of specificity of action. Rather, the toxic group should be adjacent to that area of the substrate at which attachment occurs, so that once enzyme-antimetabolite interaction has taken place, nearby sites of the enzyme could be attacked irreversibly with the formation of a covalent bond. Amino acid-mustards would be expected to exert their activity in this fashion.

Similarly, it might be hoped that compounds like 5-bis(β-chloroethyl) aminouracil[93] (VII) or 8-bis(β-chloroethyl)amino-purines[9] (VIII) (or possibly their ribonucleosides or deoxyribonucleosides) might be incorporated into nucleic acids and then, through their "mustard" groups, perhaps cause cross-linking between adjoining Watson-Crick double helices.



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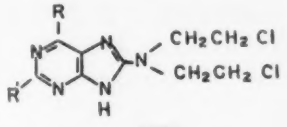
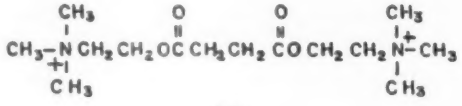


VIII.

The main problem with antimetabolites of this type would be expected to lie in the likelihood that they might undergo chemical reaction before having had time to reach the desired destination.

For a related discussion of irreversible antimetabolites the reader is referred to a recent paper by Baker.[3]

3. *Aggregate antimetabolites.* This type of compound was first postulated by Woolley, who suggested that two metabolites known to participate in a connected metabolic sequence could be converted into an antimetabolite by being united into a single molecule and proposed the above name.[37] Actually, it would seem unnecessary for two different metabolites to be united, but that it might suffice if two molecules of the same metabolite, or the same antimetabolite, were connected back to back, that is, linked in such a way that the chain between them would not interfere with attachment to receptor sites. Once an antimetabolite of this type would be attached, the probability of molecules of the metabolite dislodging it at both ends simultaneously would be rather low. If one end only were displaced, the probability of re-attachment of this end would be comparable to the probability of detachment of the other one. Succinoylcholine (IX), a dimer of acetylcholine, is one of the few examples of this type of compound; it is a widely used and very effective, neuromuscular blocking agent.[13] Recently some 8,8'-bispurinyl alkanes (X) have been synthesized[24] in the hope that such compounds might cause cross-linking between adjoining double helices of DNA and thus interfere with cell replication.



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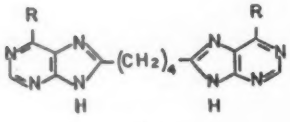


X.

It would now seem to be in order to review whether the antimetabolic approach has been or is likely to become a successful one. Although it seems likely that a research program based on the design of antimetabolites, in the long run will not produce more useful drugs than a program based on a more empirical basis, much is neglected by such a generalization. Certainly the likelihood of discovering valuable medicinal agents is particularly remote for workers synthesizing antimetabolites of compounds the functions of which are understood quite imperfectly or for workers dealing with anti-

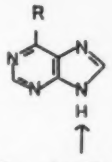
metabolites of compounds required by the host as much as by the invading cells.

However, the antimetabolite approach does have some very important advantages. While a weakly active, empirically designed compound has little or no scientific value, a weakly active, logically conceived antimetabolite may be a very valuable tool in gathering information about the metabolic functions of the compound with which it competes and about the receptor sites to which it is attached.

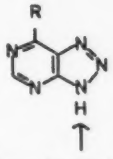
Thus, many antimetabolites do not simply compete with the compounds which they would be expected to mimic. For example, 5-fluorouracil turned out not to be a uracil antagonist in the body, but was reported as exerting its antileukemic activity by being converted to the deoxyribonucleotide and then interfering with the synthesis of thymidylic acid.[8] Similarly, 6-azauracil is converted to the ribonucleotide and interferes with orotidylic acid decarboxylase,[38] while 6-mercaptopurine has also been reported to exert its carcinostatic action at the ribonucleotide level.[8] It can be seen that such compounds are very valuable in elucidating the metabolic paths of the substrates of which they are modifications.

Furthermore, as a biochemical reaction is blocked we may see an accumulation of the product of the preceding metabolic step; this ultimately may lead to a nullification of the metabolic block. For instance, if in the reaction sequence: $A \xrightarrow{slow} B \xrightarrow{fast} C$ the step $B \rightarrow C$ is blocked, we may be able to isolate the intermediate B, which otherwise might have remained undiscovered; thus, azaserine has been very helpful in elucidating some aspects of the biosynthesis of purines.[17]

It would also seem that if the structures of metabolites are changed in such a fashion that their size remains essentially unaltered, but that the electron density of only one of the reactive groups is modified, much information about the reaction mechanisms of metabolite-enzyme interaction could be collected, at least if an isolated enzyme system were used. For example, a comparative study of the kinetics of ribonucleosidation of purines (XI) and triazolopyrimidines (XII), would be very interesting. Here, a



ribonucleosidation
XI.



ribonucleosidation
XII.

carbon is replaced by an essentially isosteric nitrogen in a position adjacent to the nitrogen where ribonucleosidation is to occur, altering the electron density of the latter in a predictable fashion. Since electron density determines the ease with which electrophilic or nucleophilic reactions may proceed, the relative rate of ribonucleosidation of isologues of this type should give us much useful information about the mechanisms involved.

By measuring the dissociation constants of enzyme-substrate complexes of the enzyme with analogs in which the electrophilic character of only one group has been altered, we can gain knowledge about the acidity or basicity of the groups on the enzyme with which binding occurs. This would be particularly true if such measurements were carried out over a wide pH range. If, on the other hand, the electron density of the reactive groups is left unaltered, but the size of the molecule or the distance between the groups is modified, information can be collected about the shape of the sites where attachment is to occur. Such an approach has been used very successfully by Nachmansohn, Wilson, and their associates, in studying the receptor sites of acetylcholine esterase with the result that it became possible to design, on a rational basis, compounds for reversing inhibition of this enzyme.[27]

It would seem that at our present state of knowledge, in which we can discuss enzyme reactions mainly in terms of what substrate is attacked rather than in terms of the reaction mechanism of the attack, antimetabolites have their greatest value in explaining the mode of action of metabolites and in collecting information about receptor sites. The chance of finding useful drugs, at present, may be no higher than if any other chemotherapeutic approach were used, but it is intellectually a great deal more satisfying and much more productive of fundamental information upon which to build future advances.

Once enzymic reaction mechanisms and receptor sites would be understood in molecular terms rather than in terms of vague generalities, rational chemotherapy might become a reality. Antimetabolites would seem to be our most valuable tool for reaching this point.

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HERBERT KAPLAN*
GERALD KLATSKIN**

Department of Internal Medicine, Yale University School of Medicine

SARCOIDOSIS, PSORIASIS, AND GOUT: SYNDROME OR COINCIDENCE?†

In the past year we have encountered three patients with coexistent sarcoidosis, psoriasis, and gout. Each of the patients exhibited the classical clinical features of all three diseases, and in each the diagnosis of sarcoidosis and psoriasis was confirmed histologically.

It is interesting to note that the skin lesion described by Jonathan Hutchinson in 1877,[1] and generally considered to be the first recorded instance of sarcoidosis, occurred in an elderly gentleman with gout. In a later report[2a] Hutchinson recalled this patient with the remark; "I was inclined to consider the skin-disease as essentially connected with the gout." At the same time he described a second case with the two diseases.[2b] To our knowledge no other authors have commented on the association between sarcoidosis and gout, and none has reported a case of coexistent sarcoidosis, psoriasis and gout.

Obviously the occurrence of three apparently unrelated but relatively common diseases in the same individual suggests a coincidence. However, the discovery in a one-year period of three patients with the triad prompted us to investigate the possibility of an interrelationship between these three diseases. To that end the clinical records of patients with well-documented sarcoidosis, gout or psoriasis were reviewed for evidence of any combination of those diseases. In addition, the serum uric acid levels were determined in two groups of patients with sarcoidosis and psoriasis, respectively.

The results of these studies, taken together with certain evidence culled from the literature, suggest that the coexistence of sarcoidosis, psoriasis, and gout may be indicative of some as yet obscure relationship among these diseases.

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* Trainee, National Institute of Arthritis and Metabolic Diseases.
** Professor of Medicine.

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CASE REPORTS

Case 1 (48-85-89). L. B., a 41-year-old white salesman, was admitted to the Grace-New Haven Community Hospital in April 1958 because of recurrent pain in the ankles and toes.

In 1950 there was a sudden onset of severe pain in the metatarsophalangeal joint of the right great toe, which subsequently became swollen, exquisitely tender and warm. A blood uric acid was said to be high, leading to the diagnosis of gout. The joint pain responded to colchicine, but recurred at six-month intervals initially, and then almost monthly. Benemid had been tried for one or two months on two occasions, but had had no effect on the frequency or severity of the attacks. A serum uric acid early in 1958 was 8.5 mg. per cent.

Since his early twenties the patient had been afflicted with psoriasis, which had appeared first on the scalp and legs, and had then gradually progressed to involve the arms, abdomen, sacrum, and groin. Many forms of topical therapy had proved ineffective.

Although the patient had never had any symptoms of cardio-respiratory disease, a routine roentgenogram of the chest in 1954 had shown a pulmonary lesion interpreted as silicosis.

There was no family history of arthritis or skin disease.

Physical examination revealed a moderately obese man who did not appear ill. Blood pressure was 140/100. Large, well-circumscribed, erythematous patches with silvery scales, considered typical of psoriasis, were present over the scalp, abdomen, groin, sacrum, and extensor surfaces of all four extremities. The lacrimal and parotid glands were not enlarged. The corneas and fundi were normal. There was no lymphadenopathy. Breath sounds were increased over both lung fields. The heart was normal in all respects. Neither the liver nor the spleen was palpable. Slight erythema and tenderness to compression were present over the right ankle. There were no other deformities of the bones or joints, and there were no tophi present.

Urinalysis, hematocrit, leukocyte and differential counts were normal. The NPN was 35 mg. per cent; the serum proteins totaled 7.83 gm. per cent, with 3.41 gm. per cent of albumin and 4.42 gm. per cent of globulin. The serum calcium was 10.2 mg. per cent, serum inorganic phosphorus 3.6 mg. per cent. The serum uric acid concentration on two occasions was 11.4 and 10.8 mg. per cent, respectively. Liver function studies revealed a serum bilirubin level of 1.18 mg. per cent, with a direct-reacting fraction of 0.41 mg. per cent; bromsulphalein retention at 45 minutes, 17.8 per cent; cephalin-cholesterol flocculation, 2+ in 24 and 48 hours; thymol-turbidity, 10.7 units; thymol flocculation, 4+; serum alkaline phosphatase, 14.2 units by the Shinowara-Jones-Reinhart modification of the Bodansky method; serum glutamic-oxalacetic transaminase, 32 units. Phenolsulfonphthalein excretion was normal as was the electrocardiogram.

X-ray examination of the chest revealed marked linear and nodular infiltration in both lung fields, most marked in the upper lobes, without evidence of hilar adenopathy. The bones of the hands and feet were normal, and there were no esophageal varices demonstrable radiographically. Pulmonary function tests revealed a low residual volume, hyperventilation, and an increase in the alveolar-arterial gradient to 18.8 mm. of mercury. First and second strength intracutaneous tuberculin (PPD) tests were negative.

The psoriasis responded to coal tar ointment and ultra-violet light therapy. A course of Benemid was prescribed, and the patient was discharged from the hospital.

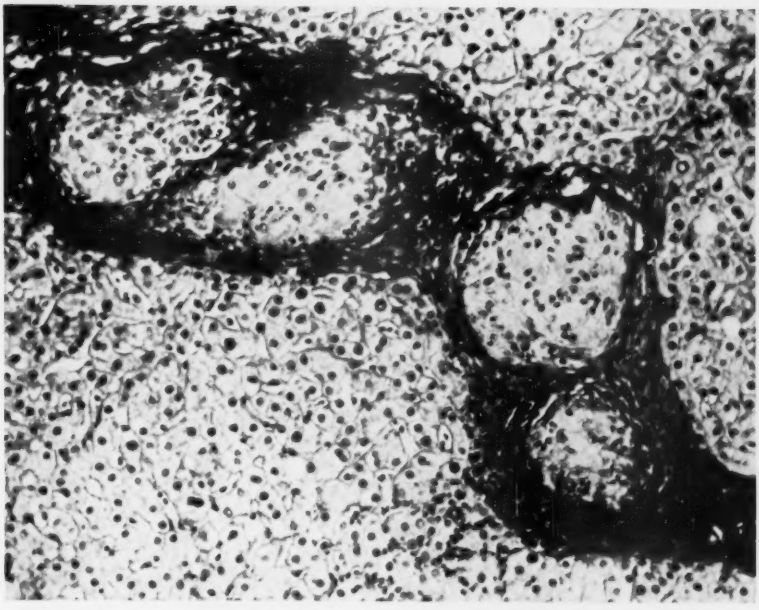

FIG. 1. Case 1. Liver biopsy specimen showing numerous noncaseating granulomata containing epithelioid and giant cells surrounded by dense collagen bundles. Masson stain, original magnification x120.

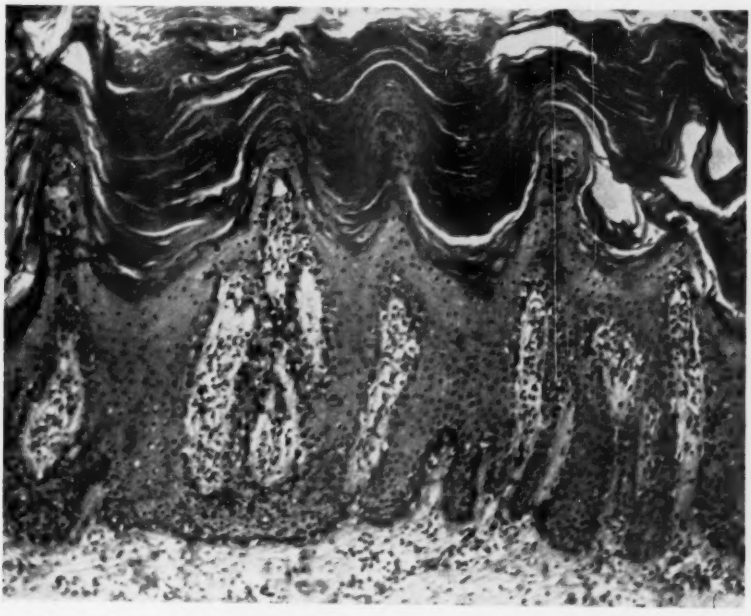

FIG. 2. Case 1. Skin biopsy specimen showing parakeratosis, elongation of the epidermal pegs with thinning of the overlying epidermis, elongation of the dermal papillae with dilatation of the capillaries, and an inflammatory reaction in the dermis, features considered typical of psoriasis. Hematoxylin and eosin stain, original magnification x60.

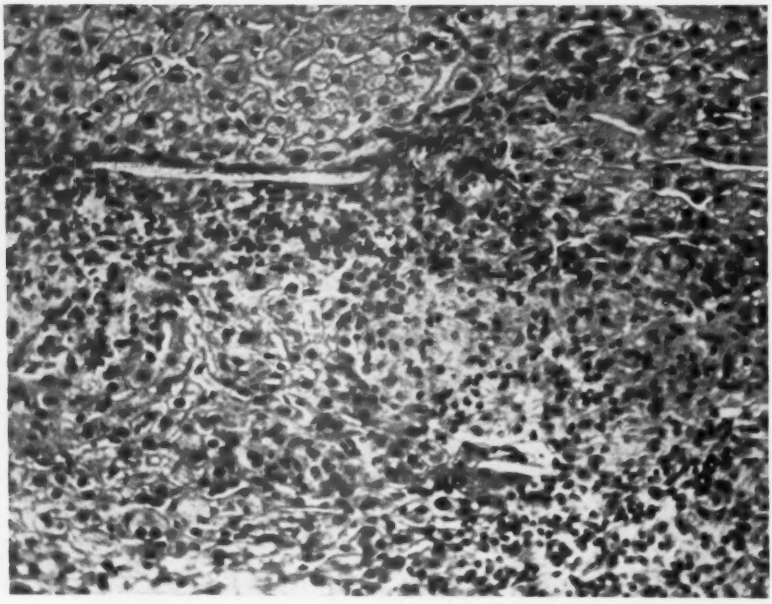

FIG. 3. Case 2. Liver biopsy specimen showing a paracentral granuloma made up of epithelioid and occasional giant cells, surrounded by a thin rim of lymphocytes. Masson stain, original magnification x120.

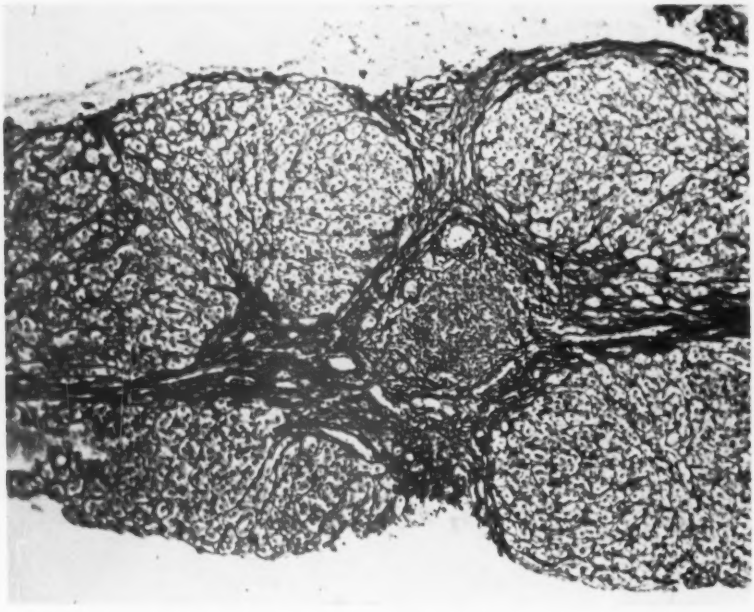

FIG. 4. Case 3. Liver biopsy specimen showing diffuse fibrosis and pseudolobule formation. In the center is a circumscribed compact mass of large monocytes, suggestive but not typical of epithelioid cells, with a paucity of reticulum fibers. Laidlaw reticulum stain, original magnification x60.

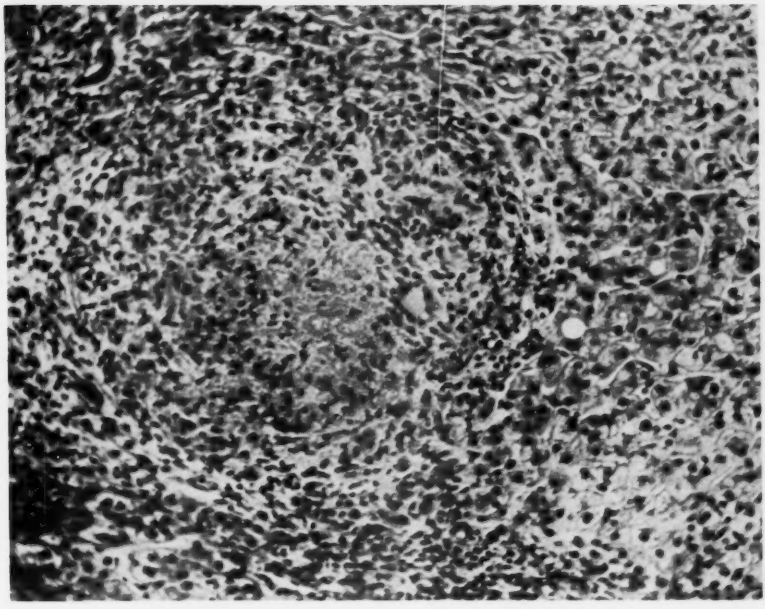

FIG. 5. Case 3. Liver biopsy specimen showing a circumscribed periportal granuloma made up of epithelioid and giant cells with a small central zone of eosinophilic necrosis and a thin rim of lymphocytes. Masson stain, original magnification x120.

One month later the patient was readmitted for a liver biopsy to establish whether or not he had sarcoidosis. He had had no joint pains since discharge, and the skin was much improved. The only new finding was parotid enlargement. The white blood cell count was 7.150 per cu. mm., with 19 per cent eosinophils. Serum calcium was 11.7 mg. per cent, while the serum inorganic phosphorus was 2.9 mg. per cent. Liver function tests and x-ray examination of the chest showed no change. A needle biopsy specimen of the liver revealed relatively normal parenchyma with numerous granulomata (Fig. 1), consisting of compact masses of epithelioid and giant cells, considered compatible with sarcoidosis. Many of the granulomata were confluent and were surrounded by collagen fibers which formed septa distorting the normal lobular architecture. In some areas the parenchyma was arranged in a nodular pattern, suggesting an early cirrhosis of granulomatous origin.

The patient was started on prednisone, 15 mg. daily, before his discharge from the hospital and was advised to continue his Benemid therapy as before.

In January 1959, eight months after starting steroid therapy, he was readmitted because of an exacerbation of his psoriasis. He had had no recurrence of gout since the institution of prednisone and Benemid therapy nine months previously. The only new finding was the presence of a palpable epitrochlear node on the right. Liver function studies showed some evidence of improvement in that bromsulphalein retention had dropped to 6.9 per cent, the cephalin-cholesterol flocculation test had become negative, and the serum alkaline phosphatase had fallen to 10.0 units. X-ray examination of the chest revealed no significant change, but pulmonary function studies showed some improvement. Again the psoriasis responded to treatment with coal tar ointment and ultra-violet light. Because of the apparent improvement in hepatic and pulmonary function, the dose of prednisone was slowly tapered.

When last seen in May 1959, the patient was taking 7.5 mg. of prednisone daily and was asymptomatic with respect to his sarcoidosis and gout; however, his psoriasis had recently flared up. Serum uric acid at this time was 4.7 per cent, and the results of liver function tests were essentially unchanged from those obtained six months previously. A biopsy specimen of the skin revealed the characteristic features of psoriasis (Fig. 2). Repeat biopsy of the liver showed essentially the same changes noted 11 months earlier, except that many of the granulomata had undergone hyalinization.

Comment. This patient had extensive psoriasis for a period of ten years, following which he developed classical, but apparently nonfamilial gout. Four years later, a routine roentgenogram of the chest disclosed the presence of asymptomatic pulmonary sarcoidosis. The possibility that the actual onset of sarcoidosis antedated the appearance of psoriasis or gout cannot be excluded, since the age of the pulmonary lesions at the time of their discovery could not be estimated. Similarly, the possibility that hyperuricemia was present before either of the other diseases had appeared cannot be discounted. Accordingly, no conclusions are warranted regarding the precise sequence in which these diseases developed.

Case 2 (C44116). C. D., a 51-year-old white university professor, was seen in February 1959, complaining of shortness of breath.

The patient had previously been admitted to the Ophthalmology Service of the Grace-New Haven Community Hospital in July 1950 because of pain in the eyes and blurring of vision bilaterally. He had had an attack of erythema nodosum one month previously. Physical examination revealed bilateral iridocyclitis. No lymphadenopathy, skin lesions, or enlargment of the salivary glands was noted. The lungs were clear, and the heart was normal except for a grade one apical systolic murmur. A soft liver edge was palpable two fingerbreadths below the right costal margin, but there was no splenomegaly. Hemogram, urinalysis, and NPN were normal. X-ray examination of the chest revealed minimal bilateral pulmonary emphysema and bilateral hilar adenopathy. A tentative diagnosis of sarcoidosis was made, following which oral cortisone was administered for a period of ten days resulting in improvement in the eye symptoms.

Two months later the patient developed bilateral secondary glaucoma, necessitating his readmission for iridectomy.

One year later, in June, 1951, he was admitted to the Medical Service because of a recurrence of his uveitis, and the appearance of a nonproductive cough associated with generalized myalgia and arthralgia. Except for the signs of bilateral uveitis and the previously described slight hepatomegaly, physical examination revealed no abnormalities. The leukocytes numbered 4,200 per cu. mm. with a normal differential count. The results of liver function tests, including serum bilirubin, bromsulphalein retention, cephalin-cholesterol flocculation, thymol-turbidity and serum alkaline phosphatase, were all normal. Serum proteins totaled 6.53 gm. per cent, with 3.38 gm. per cent of albumin and 3.15 gm. per cent of globulin. The serum concentrations of calcium and inorganic phosphorus were 10.5 and 3.9 mg. per cent, respectively. Tuberculin (PPD, first and second strengths), coccidioidin and histoplasmin skin tests, and a brucella agglutination test were all negative. Gastric aspirate inoculated into a guinea pig was negative for tubercle bacilli. X-ray examination of the chest showed bilateral hilar adenopathy without evidence of pulmonary infiltration. A needle biopsy specimen of the liver revealed numerous noncaseating granulomata composed of compact aggregates of epithelioid cells and a few giant cells (Fig. 3). Acid-fast stains for tubercle bacilli were negative. These findings were considered compatible with the clinical diagnosis of sarcoidosis. An eight-day course of intravenous ACTH resulted in a remission of the ocular symptoms, which have not recurred in eight years.

In 1955, five years after the onset of his sarcoidosis, the patient noted the insidious onset of dyspnea on exertion. Shortly thereafter, a roentgenogram of the chest revealed bilateral pulmonary infiltrates indicating extension of the sarcoidosis to the lungs. Over the succeeding three years the pulmonary lesions increased, but there was little change in the severity of the respiratory symptoms until late in 1958 when dyspnea increased rapidly and became disabling.

In June 1956 the patient was awakened one night by a severe pain in the metatarsophalangeal joint of the right great toe, which became red and hot, and swelled to twice normal size. The erythema about the base of the toe extended to the entire dorsum of the foot. Colchicine was administered by his personal physician, resulting in prompt relief. Unfortunately, the blood level of uric acid was not determined at this time, and subsequent x-ray examination of the toe revealed no abnormalities.

Two years later, in July 1958, the patient was again awakened by similar pain in the right great toe and obtained prompt relief with colchicine. Between 1957 and 1959 he experienced several episodes of pain and swelling of the knees, without associated redness or heat, of approximately two weeks' duration each. Colchicine was not tried

because the patient did not regard these as attacks of gout. However, small doses of salicylate afforded some relief.

Typical psoriatic lesions made their appearance in December 1958. Well-circumscribed, erythematous, oval patches covered with silvery scales were noted first on the flexor surface of the right forearm, and then over the elbows, calves, and scalp.

There was no family history of arthritis or skin disease.

Physical examination in February 1959 revealed a husky middle-aged man who did not appear ill. There were typical psoriatic patches over the scalp, both elbows, and both calves. Keyhole postoperative deformities of both pupils were present, but there were no signs of active uveitis or conjunctivitis. The parotid and lacrimal glands were not enlarged, and there was no evidence of lymphadenopathy. The lungs were clear. The heart was of normal size, had a normal rhythm, except for occasional runs of tachycardia, and showed a loud blowing precordial systolic murmur which radiated to the neck, axilla, and back. The liver edge was felt at the right costal margin, but the spleen was not palpable. The right knee was slightly swollen, but contained no obvious fluid, and was not tender. The toes were normal and there were no tophi.

The volume of packed red cells was normal. Leukocytes numbered 3,400 per cu. mm. with 12 per cent eosinophils. The urine contained a trace of albumin, but was otherwise normal. The NPN was 41 mg. per cent, and phenolsulfonphthalein excretion was normal. The serum uric acid concentration was 4.2 mg. per cent. The levels of serum calcium and inorganic phosphorus were 10.0 and 3.3 mg. per cent, respectively. Serum proteins totaled 7.54 gm. per cent, with 3.44 gm. per cent of albumin and 4.10 gm. per cent of globulin. Liver function tests revealed the following: serum bilirubin, direct-reacting, 0.25 mg. per cent, total, 1.2 mg. per cent; bromsulphalein retention at 45 minutes, 18 per cent; cephalin-cholesterol flocculation negative; serum alkaline phosphatase, 1.8 S—J—R units; serum glutamic-oxalacetic transaminase, 38 units; serum lipids, normal. A study of pulmonary function demonstrated a low residual volume and a high alveolar-arterial oxygen gradient. An intracutaneous tuberculin test (second strength PPD) was negative. X-ray examination of the chest revealed bilateral hilar adenopathy and marked fibrosis of both upper lobes. The histological features of a skin biopsy specimen were consistent with the diagnosis of psoriasis.

Because of the impaired pulmonary function, the patient was started on prednisone, 60 mg. daily. After one month of therapy his respiratory symptoms were mueh improved, and there was a concomitant increase in the maximum breathing capacity from 69.7 to 81.6 liters per minute.

Comment. In this case sarcoidosis was clearly the first of the triad of diseases to appear. This was intermittently active and slowly progressive over a period of six years, affecting the eyes, lungs, hilar nodes, and liver, following which attacks of acute gout developed. Two years later, coincident with a rather sudden increase in pulmonary symptoms, presumably due to rapid spread of the granulomatous process in the lung, psoriatic lesions appeared. It is noteworthy that, as in Case 1, there was no family history of gout. However, the clinical features and prompt response to colchicine were typical of the disease. The normal blood uric acid level observed during a symptom-free period was not considered sufficient evidence to exclude the diagnosis of gout, since normal values are known to

occur occasionally.[3] Whether the attacks of painful swelling of the knees were gouty in nature, or were manifestations of the nonspecific arthritis sometimes seen in sarcoidosis,[4] is uncertain.

Case 3 (36-95-75). J. M., a 59-year-old negress, was admitted to the Grace-New Haven Community Hospital in March 1959 complaining of painful calves.

In November 1954 the patient was seen in the Dermatology Clinic because of the recent development of scattered pruritic and lichenified patches on the calves, thighs, and arms. Three months later, new lesions appeared on both elbows and, over the next year, these spread to involve the entire body, except for the palms and soles. In addition to being lichenified, the lesions were covered with silvery scales suggesting psoriasis.

In February 1956 the patient had the first of many hospitalizations for treatment of her skin condition. At this time the liver was found to be palpable two fingerbreadths below the right costal margin. Liver function tests, including serum bilirubin, cephalin-cholesterol flocculation, thymol turbidity, and serum alkaline phosphatase, yielded normal results except for a bromsulphalein retention of 21 per cent at 45 minutes. The NPN and the serum proteins, calcium, and inorganic phosphorus also were normal. X-ray examination of the chest revealed no abnormalities.

The pruritus abated following a week of oral prednisone but returned when treatment was stopped. The skin lesions showed no change and were unaffected by topical therapy of several types.

In March 1957 biopsy of the skin revealed histological features considered typical of psoriasis. Serum proteins at this time totaled 7.36 gm. per cent, with 2.27 gm. per cent of albumin and 5.09 gm. per cent of globulin.

During the succeeding two years the skin showed little change except for transient improvement following a course of oral prednisone, ultraviolet radiation, and topical coal tar ointment.

In September 1958 the patient had an attack of thrombophlebitis. This recurred in March 1959, necessitating hospitalization. Because of the incidental finding of the previously noted hepatomegaly and bromsulphalein retention, which were still evident, we were asked to see the patient in consultation. Review of her history revealed additional points of interest not previously noted. For a number of years starting in childhood the patient had had recurrent attacks of left-sided renal colic and hematuria, and had been relieved of these symptoms following a nephrectomy with the removal of renal calculi. Nine years previously, at the age of 50, she had had a panhysterectomy because of recurrent spontaneous abortions. A year later, in 1951, she had had her first attack of what was almost certainly acute gout, although the diagnosis had never been suggested. Following this the attacks had recurred several times a year. Typically they began abruptly with a gnawing pain in the metatarsophalangeal joint of the right great toe, which then increased in intensity and was followed by swelling, redness, and exquisite tenderness. Usually the attack lasted for about a week during which time the patient could not wear a shoe, bear the weight of her bedclothes, or get around without crutches. Salicylates in small doses provided slight and only temporary relief. She had never tried colchicine. In addition to her attacks of acute gout, the patient had been troubled for a period of four years with a chronic migratory arthritis of the knees, elbows, wrists, and interphalangeal joints. This was characterized by dull pain, occasionally accompanied by swelling. During the past three years the patient had also had attacks of pain in both eyes accompanied by tearing bilaterally, and by blurring

of vision on the right. These episodes had occurred approximately twice yearly and had lasted up to a week.

There was no family history of arthritis or skin disease. The patient had never used alcohol to excess, and her diet had been adequate.

Physical examination revealed an obese negress who did not appear ill. Her blood pressure was 180/90. The skin lesions were as previously described. On both sides the lacrimal glands were greatly enlarged. The corneas and fundi were normal, and, on slit-lamp examination, no evidence of recent or old uveitis was noted. The parotids were not enlarged, and there was no lymphadenopathy. The lungs were clear. The heart was normal except for a rate of 102 per minute. A firm, round, slightly tender liver edge was palpable three fingerbreadths below the right costal margin in the midclavicular line. The spleen was not felt. There was tenderness to palpation, increased heat, and slight swelling over both calves. Except for bilateral Heberden's nodes, the joints appeared normal. There were no tophi.

Urinalysis, the NPN, and phenolsulfonphthalein excretion were normal. Leukocytes numbered only 3,800 per cu. mm., but the differential count was normal. The concentrations of serum calcium and inorganic phosphorus were 11.9 and 4.9 mg. per cent, respectively. Serum proteins totaled 7.61 gm. per cent, with 2.79 gm. per cent of albumin and 4.82 gm. per cent of globulin. Liver function studies showed the following: serum bilirubin, direct-reacting, 0.70 mg. per cent, total, 1.4 mg. per cent; bromsulphalein retention at 45 minutes, 2.3 per cent; cephalin-cholesterol flocculation, negative at 24 and 48 hours; thymol turbidity, 4.3 units; serum alkaline phosphatase, 7.5 S—J—R units; serum glutamic-oxalacetic transaminase, 59 units. The sensitized sheep-cell test for serum rheumatoid factor was negative. Test for brucella agglutinins was negative. An intracutaneous tuberculin test with PPD was negative in the first strength and positive in the second strength. Culture of gastric aspirate for tubercle bacilli was negative. Studies of pulmonary function revealed no abnormalities. A roentgenogram of the chest was normal except for left ventricular enlargement. X-ray studies of the knees showed minimal osteoarthritic changes bilaterally, and those of the hands and feet only early osteoporosis. A roentgenogram of the abdomen did not demonstrate calcification in the region of the remaining kidney.

Needle biopsy of the liver (Fig. 4) revealed diffuse portal fibrosis with pseudolobule formation. Some of the connective tissue septa contained numerous lymphocytes and circumscribed collections of large monocytes containing abundant cytoplasm and large nuclei. In one periportal area there was a large granuloma made up of a compact mass of epithelioid and giant cells, in the center of which there was a small zone of eosinophilic necrosis (Fig. 5). An acid-fast stain of several sections failed to demonstrate tubercle bacilli. The histological picture was considered consistent with a granulomatous cirrhosis due to sarcoidosis.

Comment. As a result of our experience with Cases 1 and 2, the association of psoriasis with joint symptoms suggested the possibility that the patient had gout and that her hepatomegaly was due to sarcoidosis, diagnoses that had not been considered previously and that were ultimately confirmed.

Both the history and the hyperuricemia were consistent with gout. As in the previous two cases, there was no family history of the disease. Although gout is rare in negro females, it has been reported by others.[5,6] It

may be of significance that the gout appeared shortly after panhysterectomy, since the occurrence of gout in women is more common after the menopause.[7] Conceivably, the two episodes of thrombophlebitis also were related to the gout in view of previous reports of this association.[8]

The diagnosis of sarcoidosis was based on the biopsy findings in the liver and was supported by the presence of enlarged lacrimal glands, the suggestive history of uveitis, the patient's race and birthplace in the rural Southeast, and the finding of hyperglobulinemia and hypercalcemia. Conceivably, the renal calculi present earlier in life were also related to her sarcoidosis. Although the predominence of granulomatous lesions in the liver with the production of a cirrhotic picture is unusual in sarcoidosis, one of us (GK) has reported one such case[9] and has seen several others.

It is reasonably clear that the gout antedated the psoriasis by three years. Hepatomegaly, later shown to be due to sarcoidosis, was not discovered until two years later. However, it is not known when the hepatomegaly developed, since there is no record of a prior abdominal examination. The history of renal calculi very early in life, taken together with the later finding of slight hypercalcemia, suggests that sarcoidosis could have been present in childhood. Also consistent with the hypothesis that the disease was of very long standing was the advanced degree of fibrosis of apparent granulomatous origin found in the liver.

OBSERVATIONS

In a search for possible interrelationships between sarcoidosis, psoriasis, and gout, the clinical records of patients previously hospitalized with each of these diseases were reviewed for evidence of the coexistence of the others. Included were 73 successive cases of histologically confirmed sarcoidosis, and 100 random selected cases each of well-documented psoriasis and gout. In looking for evidence of sarcoidosis in the latter two groups, particular attention was paid to the condition of the skin and the eyes, the size of the lymph nodes, liver, and spleen, the x-ray findings in the chest, the levels of serum globulin, calcium and alkaline phosphatase, and any history of antecedent ocular inflammation or salivary gland enlargement.

In addition, 25 of the sarcoidosis cases and 45 of the psoriasis cases were re-investigated for hyperuricemia, employing the Brown modification[10] of the Folin method for uric acid. Serum concentrations in excess of 6.0 mg. per cent in males and 5.0 mg. per cent in females are considered abnormal in this laboratory.

Sarcoidosis. None of the 73 patients with sarcoidosis exhibited psoriasis or the features of classical gout. However, six of the 25 whose blood uric

TABLE 1. OBSERVATION ON 73 CASES OF SARCOIDOSIS (EXCLUSIVE OF THREE PATIENTS WITH COEXISTENT PSORIASIS AND GOUT)

Case no.	*Sex*	*Serum uric acid (mg.%)*	*NPN (mg.%)*	*PSP excretion (%)*	*Serum Ca (mg.%)*	*Serum P (mg.%)*	*Joint symptoms*	*Skin lesions*
1	M	*7.8**	*53*	..	9.4	4.4	+	Seborrheic dermatitis
2	M	*7.4*	16**	..	9.9	3.0	0	0
3	F	*6.9*	30	57	9.4	2.7	+	0
4	F	*6.3*	30	..	9.8	4.7	0	0
5	F	*5.5*	39	54	*13.8*	3.2	0	0
6	F	*5.4*	36	..	9.0	4.1	0	0
7	M	5.7	32	..	*11.6*	3.6	0	0
8	M	5.7	19**	..	10.5	4.5	0	0
9	M	5.6	27	..	10.3	3.6	0	0
10	M	5.4	10**	..	11.1	2.3	0	Seborrheic dermatitis
11	F	5.0	11**	..	...	..	0	0
12	F	5.0	25	..	10.4	4.7	0	0
13	F	4.7	32	..	9.4	4.3	0	0
14	F	4.6	30	..	9.7	3.7	+	Seborrheic dermatitis
15	F	4.6	30	..	10.2	5.0	0	0
16	F	4.4	37	..	10.2	3.6	+	0
17	F	4.4	33	..	*11.4*	4.3	+	0
18	F	4.4	12**	..	9.9	2.7	0	0
19	M	4.3	31	..	10.5	2.7	0	0
20	M	4.0	35	..	10.2	3.4	0	0
21	M	4.0	7**	..	10.2	4.5	0	0
22	F	3.9	40	..	10.0	3.3	0	Sarcoidosis
23	M	3.7	30	..	10.3	3.0	0	0
24	M	3.7	27	..	9.0	4.5	0	0
25	F	3.3	27	..	...	..	0	0
26	M	..	30	..	9.5	3.4	0	Lichen planus
27	M	..	33	..	9.4	4.4	+	0
28	F	..	27	..	...	..	+	0
29	M	..	28	..	9.9	2.8	+	0
30	F	..	23	..	10.2	3.6	+	Sarcoidosis
31-73	M,F	..	..	..	...	..	0	Seborrheic dermatitis (1) Vitiligo (1) Acne rosacea (1) Dermatitis, type ? (1)

* Abnormal values italicized.
** BUN.

acid was determined had hyperuricemia (Table 1). In only one of these (No. 1) was there azotemia. Another (No. 5) had hypercalcemia, but renal function was unimpaired. Hence, 20 per cent of the group tested had an otherwise unexplained elevation of serum uric acid, an incidence considerably higher than that previously reported in nongouty individuals.[7, 11]

Migratory joint pain, often accompanied by swelling, was reported as a significant symptom in nine of the 73 cases investigated. Two of these (No. 1 and 2) had hyperuricemia, but the character of the joint manifestations did not suggest gout in either. In No. 1, the sensitized sheep-cell test for rheumatoid arthritis was positive, while in the remaining cases the joint symptoms were interpreted as manifestations of the sarcoidosis.

Psoriasis. In reviewing the records of 100 cases of psoriasis, no unequivocal instances of coexistent sarcoidosis or gout were encountered. However, there was suggestive evidence of these diseases in several (Table 2).

Sixteen of the group had arthritis. Although the latter was not typical of gout in any, in Case 9 it was accompanied by a hyperuricemia of 6.8 mg. per cent, and in Case 26 it began acutely in one of the great toes, although subsequently other joints were affected, and the serum uric acid was found to be normal. Ten of the remaining 14 patients with joint symptoms had what was regarded as classical rheumatoid arthritis.

Although the diagnosis of sarcoidosis was not established in any of the group, 11 patients exhibited roentgenographic signs of otherwise unexplained pulmonary fibrosis, significant hyperglobulinemia, hepatomegaly, or splenomegaly, either alone or in combination (Table 2). Case 15 was of particular interest in that the patient had psoriasis, slight hyperuricemia, pulmonary fibrosis, recurrent corneal ulcers, marked hyperglobulinemia (5.08 gm. per cent), and, on two occasions, was found to have small hepatic granulomata in needle biopsy specimens. Sarcoidosis was considered a distinct possibility, but the hepatic granulomata were deemed too small to warrant an unequivocal diagnosis. Liver biopsy in three of the other patients with hepatomegaly failed to reveal granulomas. In the remaining cases with hepatomegaly, hyperglobulinemia, or pulmonary fibrosis the possibility of sarcoidosis had not been considered, and, hence, had not been investigated.

Eighteen of the 47 patients with psoriasis whose blood uric acid was determined were found to have hyperuricemia (Table 2). Renal function was impaired in Cases 10 and 13, and was not investigated in five others, but was normal in the remainder, so that in at least 20 per cent of the group the hyperuricemia was otherwise unexplained.

TABLE 2. OBSERVATIONS ON 100 CASES OF PSORIASIS (EXCLUSIVE OF THREE CASES WITH COEXISTENT SARCOIDOSIS AND GOUT)

Case no.	Sex	Serum uric acid (mg.%)	NPN (mg.%)	Arthritis	Chest x-ray	Hepatomegaly	Splenomegaly	Eye signs	Serum proteins (gm.%) Total	Alb	Glob
1	M	10.1	40	0	..	0	0	0	N**	N	N
2	M	8.0	28	0	Neg.	0	0	0	N	N	N
3	M	7.6	30	0	Neg.	0	0	0	N	N	N
4	M	7.4	27	0	Neg.	0	0	0	N	N	N
5	M	7.2	..	0	..	0	0	0	...	...	...
6	M	7.2	25	0	..	0	0	0	...	...	...
7	M	7.1	17	0	..	0	0	0	...	...	...
8	M	6.9	35	0	Neg.	0	0	0	N	N	N
9	M	6.8	36	+	..	0	0	0	N	N	N
10	F	6.7	14*	0	..	0	0	0	...	...	...
11	F	6.5	23*	0	Neg.	+	0	0	...	...	...
12	F	6.5	..	0	Neg.	0	0	0	...	...	...
13	F	6.5	22*	0	..	0	0	0	...	...	...
14	F	6.3	..	0	Neg.	0	0	0	...	...	...
15	M	6.1	30	0	Pulmonary fibrosis	0	0	Recurrent corneal ulcers	7.60	2.52	5.08
16	M	6.1	..	0	..	0	0	0	...	...	...
17	F	5.9	9*	0	..	0	0	0	...	...	...
18	F	5.1	..	0	Neg.	+	0	0	...	...	...
19	M	5.8	..	0	Neg.	0	0	0	...	...	...
20	M	5.3	..	0	Neg.	0	0	0	N	N	N
21	F	5.0	..	0	..	0	0	0	...	...	...

Case no.	Sex	Serum uric acid (mg.%)	NPN (mg.%)	Arthritis	Chest x-ray	Hepatomegaly	Splenomegaly	Eye signs	Serum proteins (gm.%) Total	Alb	Glob
22	M	4.7	..	0	..	0	0	0	...	...	...
23	F	4.6	..	0	Neg.	0	0	0	...	...	...
24	F	4.6	..	0	Neg.	0	0	0	N	N	N
25	F	4.5	..	0	Neg.	0	0	0	...	...	...
26	F	4.3	28	+	Neg.	0	0	0	...	...	...
27	M	4.2	38	+	Neg.	0	0	0	7.17	3.08	*4.09*
28	M	4.2	..	0	Neg.	0	0	0	...	...	...
29	F	4.2	..	0	..	0	0	0	...	...	...
30	F	4.1	..	0	..	0	0	0	...	...	...
31	F	4.0	..	0	Neg.	0	0	0	...	...	...
32	F	4.0	..	0	Neg.	0	0	0	6.13	*2.87*	3.26
33	F	4.0	..	0	Neg.	0	0	0	...	...	...
34	M	4.0	35	0	Neg.	0	0	Corneal ulcers	...	...	...
35	M	3.9	..	0	..	0	0	0	...	...	...
36	M	3.9	..	0	..	0	0	0	...	...	...
37	M	3.9	..	0	Neg.	0	0	0	N	N	N
38	F	3.8	..	0	..	0	0	0	...	...	...
39	F	3.8	..	0	..	0	0	0	...	...	...
40	F	3.6	..	0	Neg.	0	0	0	...	...	...
41	F	3.4	..	0	..	0	0	0	...	...	...
42	M	3.3	..	+	Neg.	0	0	0	...	...	...
43	F	3.2	..	0	..	0	0	0	...	...	...
44	F	3.1	17*	+	Neg.	0	0	0	...	...	...
45	F	3.0	28	+	Neg.	0	0	0	...	...	...

46	F	3.0	.	+	Neg.	0	0	0	...	...	...
47	F	3.0	36	0	Neg.	0	0	0	...	...	...
48	F	..	..	+	..	0	0	0	...	...	...
49	F	..	..	+	Neg.	0	0	0	...	...	...
50	M	..	..	+	Neg.	0	0	0	*5.67*	*2.08*	*3.59*
51	F	..	..	+	Bronchiectasis	0	0	0	...	...	...
52	F	..	..	+	Neg.	0	0	0	*8.88*	4.13	*4.75*
53	M	..	..	+	Neg.	0	0	0	N	N	N
54	M	..	..	+	Neg.	0	0	0	...	...	...
55	M	..	..	+	Pulmonary fibrosis	0	0	0	...	...	...
56	F	..	..	+	Neg.	0	0	0	...	...	...
57	F	..	..	0	..	0	0	0	7.98	*3.22*	*4.76*
58	M	..	..	0	Neg.	+	0	0	7.80	*3.60*	*4.20*
59	M	..	..	0	..	+	0	0	...	...	...
60	F	..	..	0	Neg.	+	+	0	...	...	...
61	M	..	..	0	..	+	0	0	7.88	3.84	*4.04*
62	M	..	..	0	Pulmonary fibrosis	0	0	0	6.30	*2.70*	3.60
63	F	..	..	0	Inactive tuberc.	0	0	0	7.81	*2.08*	*4.73*
64–77	M	..	..	0	Neg. (9)	0	0	0	N (5)	N (5)	N (5)
78–100	F	..	..	0	Neg. (16)	0	0	0	N (9)	N (9)	N (9)

Abnormal values italicized.

* BUN.

** N = normal.

Gout. In none of the 100 cases of gout investigated was there skin lesions suggestive of either sarcoidosis or psoriasis. However, two of the group had pulmonary fibrosis and one had hypercalcemia of undetermined etiology. Although there was no obvious collateral evidence of sarcoidosis in these cases, the diagnosis had not been considered at the time of hospitalization, and, hence, had not been adequately excluded by appropriate studies.

REVIEW OF THE LITERATURE

Although there are no previous reports of coexistent sarcoidosis, psoriasis, and gout, the literature contains references to every other possible combination of these diseases, including sarcoidosis and psoriasis, sarcoidosis and gout, and psoriasis and gout.

Sarcoidosis and psoriasis. The classical skin lesions of sarcoidosis are readily differentiated from those of psoriasis on the basis of their appearance, distribution, and histological features. However, in their monograph on sarcoidosis, Longcope and Freiman[13] describe a form of cutaneous sarcoidosis that resembles psoriasis and cite one case in their own series (No. 16) and another reported by Combes.[18] Since in neither of these patients were the characteristic granulomata of sarcoidosis demonstrated in the skin, the possibility of coexistent sarcoidosis and psoriasis cannot be excluded. A search of the literature for cases with similar skin lesions in which typical sarcoidal lesions were demonstrated proved fruitless. However, one report of coexistent sarcoidosis and psoriasis was uncovered.[14] Whether the paucity of references to the combination of sarcoidosis and psoriasis reflects its rarity, or is attributable to the failure of writers on sarcoidosis to report the occurrence of so common and apparently unrelated a disorder as psoriasis, is uncertain.

The conjunctivitis, corneal ulceration and iritis that are known to occur in the course of psoriasis[15–17] are reminiscent of the ocular complications of sarcoidosis. However, the absence of collateral evidence of sarcoidosis in such cases, and the fact that the only lesions examined have not been granulomatous in nature,[17] make it highly improbable that the eye manifestations in the two diseases share a common pathogenesis.

Lorincz[18] has suggested that psoriasis may represent an inherited pattern of reaction in the skin that may be elicited by a variety of etiological factors. Hence, if there is any connection at all between psoriasis and sarcoidosis, it is conceivable that the latter may occasionally serve as a nonspecific stimulus in provoking the former. An analogous situation is seen in the well-known association between sarcoidosis and erythema

nodosum,[39] another skin disorder that is neither granulomatous in nature nor specifically related to sarcoidosis.

The alternative possibility, that psoriasis may occasionally give rise to sarcoidosis, or that, in some instances, both diseases are due to the same etiological factor, cannot be excluded with certainty.

Sarcoidosis and gout. In addition to Hutchinson's two cases,[1, 2] only one other instance of gout[20] was encountered in a review of over 90 cases of sarcoidosis associated with arthritis. Reports of hyperuricemia in sarcoidosis are equally scarce.[21, 22, 58] However, with the exception of the present investigation, the blood uric acid has not been studied in any large group of patients with sarcoidosis. Indeed, even in the cases with arthritis reviewed by us, it was determined in only two.[24, 25] Admittedly, the transient and migratory character of the usual joint symptoms[4, 26–29] and the presence of granulomatous lesions in the synovial membranes of patients with sarcoidosis[4] are inconsistent with gout. Nevertheless, considering the fact that the arthritis may be limited to a single joint,[19, 24] and that the radiographic bone changes in sarcoidosis and gout may be indistinguishable,[19] it is conceivable that, if the possibility of gout were investigated more often, the incidence would prove to be higher than previous reports indicate.

A variety of inflammatory disorders of the eye, including uveitis and chorioretinitis, are known to occur in patients with gout.[31–33] Although these are similar to the ocular manifestations of sarcoidosis, they appear to be related to the deposition of urates rather than to a granulomatous reaction.

Obviously, gout is such a common disorder that its mere coexistence with sarcoidosis in a few instances cannot be considered adequate evidence of a relationship between these diseases. However, our finding of hyperuricemia in 20 per cent of an unselected group of 25 patients with sarcoidosis is more suggestive. One possibility to be considered is that the hyperuricemia was a manifestation of the renal dysfunction known to occur in patients with sarcoidosis as a consequence of complicating hypercalcemia[34] or granulomatous involvement of the kidney.[35] This appears unlikely, since all but one of the cases with hyperuricemia had normal renal function, and only one exhibited hypercalcemia, although admittedly subtle changes in tubular function were not excluded. An alternative possibility is that the hyperuricemia was due to an acceleration of purine metabolism of the type seen in leukemia, dependent in this instance on the excessive proliferation of the reticuloendothelial cells from which sarcoid granulomata stem.

Psoriasis and gout. Of the possible combinations of sarcoidosis, psoriasis, and gout, only that of psoriasis and gout has been reported with any degree of frequency. In one large series of patients with gout totaling 520,[36] psoria-

sis was present in 4.0 per cent, an incidence considerably in excess of that found in the general population, which is said to range between 0.27 and 1.4 per cent.[37, 38] Although these data suggest a relationship between psoriasis and gout, the specificity of this relationship is in doubt, since the incidence of psoriasis has been found to be equally high in rheumatoid arthritis and only slightly less so in osteoarthritis.[39]

Comparable data on the incidence of gout in psoriasis are not available. However, hyperuricemia has been reported in a high proportion of patients with psoriasis, being present in 35 per cent of 307 cases studied by two groups of investigators.[40, 41] Our own observations confirm these reports, but contradictory evidence, based on the study of a smaller number of patients, has been presented by others.[42]

If, as these data suggest, the incidence of hyperuricemia in psoriasis is as high as 35 per cent, it is hardly likely that it is attributable exclusively to chance alone, to unrecognized renal insufficiency, or to the use of agents known to raise the blood uric acid level.[43–49] One possibility that merits consideration is that the hyperuricemia is the consequence of an increase in purine metabolism, as in leukemia, related in this instance to the greatly accelerated proliferation of epidermal cells known to occur in psoriasis.[19] The fact that there is no correlation between the blood uric acid concentration and the severity of the psoriatic lesions[42] would appear to be contradictory. However, a similar discrepancy may be seen in leukemia, where the uric acid concentration does not necessarily parallel the number of leukocytes in the peripheral blood.

COMMENT

The evidence cited suggests some possible relationships between sarcoidosis, psoriasis, and gout, but does not settle the question of whether their coexistence in the three cases reported was a chance occurrence or the consequence of an interaction among these diseases.

Considering the striking differences in their pathogenesis and morphological features, and the variations in their time of onset, it is unlikely that all three diseases were initiated or precipitated by a single stimulus. The alternative possibility, that one of them provoked the others, appears more reasonable in view of the interrelationships previously discussed. The fact that the three diseases occurred in a different sequence in each case (psoriasis, gout, and sarcoidosis in Case 1; sarcoidosis, gout, and psoriasis in Case 2; gout, psoriasis, and sarcoidosis in Case 3), would appear to be contradictory. However, as previously indicted, it was not possible to date the onset of sarcoidosis or hyperuricemia with accuracy, so that no con-

clusions regarding the precise sequence of events in these cases are warranted. Obviously only a careful search for evidence of the triad in future cases of sarcoidosis, psoriasis, and gout can establish whether or not there is any relationship among these diseases.

SUMMARY

Three cases of coexistent sarcoidosis, psoriasis, and gout are presented. Evidence, based on a review of the literature and an investigation of patients with each of these diseases seen in the same hospital over a five-year period, is cited in support of the view that the occurrence of all three diseases in these individuals may not have been due to chance alone.

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ALVIN E. FRIEDMAN-KIEN*

Department of Anatomy, Yale University School of Medicine

DOUBLE GALL BLADDERS IN MAN: A CASE REPORT

The frequency of surgical exposure of the bile ducts and gall bladder has made the surgeon increasingly aware of the anatomic variations in these structures and their blood supply. The double gall bladder in man is a rare anomaly. Of the 103 previous reports of double gall bladders that are available to the author, only 68 occurrences of this anomaly were actually proved at operation or autopsy.[2, 14, 16] Since cholecystography was introduced by Graham and Cole in 1924, 44 cases have been detected radiographically; however, only 9 (of these) were actually confirmed by anatomical examination.[1, 16] It was stated in one article in which 36 cases were reviewed that 27 of these were reported by surgeons.[13] The nine remaining cases were autopsy and dissecting room observations. A few cases have been reported where the diagnosis was made by x-ray but was not confirmed by operative exposure. In one of these a congenitally folded gall bladder simulated a double gall bladder by cholecystography.[1]

The standard textbooks of anatomy and surgery rarely mention anomalies of the biliary tract, despite the considerable number of case reports and reviews.[7, 9, 13] As many as 47.25 per cent of patients requiring biliary surgery demonstrated deviations from the "normal" biliary tract anatomy described in textbooks.[10] Similarly, the "normal" anatomy of cystic and hepatic arteries was present in only 32.0 per cent of 200 cases at surgery.[8]

The earliest available records of gall bladder anomalies in animals appeared in the cuneiform writings of the Babylonian civilization dealing with religious symbolism.[12] Pliny described a double gall bladder in an animal sacrificed in 31 B.C. during the celebration of Augustus Caesar's victory over Antony and Cleopatra. During the ninth century A.D. Joseph Caro's "Shulhan Aruch," the authoritative codification of Jewish dietary laws, mentioned nine specific rules concerning animals possessing duplication of the gall bladder which rendered such meat unfit for human consumption.[4] Anomalous gall bladders are more common in animals than man.[4] Dissection of 10,000 animals revealed 23 duplications of the gall bladder in 2,600

* Fourth-year medical student; Chief Prosector in Anatomy.

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cats, a higher ratio of 1 out of 28 in calves, 1 out of 85 sheep and 1 in 198 pigs. Between 1674 and 1926, 20 double gall bladders in man were reported.[4] Only 2 authentic double gall bladders were detected by the dissections of 9,221 human cadavers.[4] The first surgical diagnosis of a double gall bladder was reported in 1911, but it was not until 1929 that the initial diagnosis was made by cholecystography.[9] Three cases of accessory gall bladders were observed among 10,000 patients at surgery prior to 1926.[4]

Three anatomical varieties of double gall bladder (vesica fellea duplex) have been described by Boyden.[4] The *"Y"-type* is characterized by two cystic ducts joining to form a common cystic duct, which in turn enters the common bile duct. The "ductular" or *"H"-type* double vesicle possesses completely separate cystic ducts. Either of the ducts may enter the right or left hepatic ducts, or both may enter the common bile duct independently. Only one human case of a "trabecular" type duplicate gall bladder has been observed, although it has been seen more frequently in animals.[4,9] The cystic duct of the primary gall bladder joined the common bile duct as usual; the cystic duct of accessory organ entered directly into the liver substance.

Triplication of the gall bladder has been seen three times in man. In each case the individual vesicles possessed separate cystic ducts which entered the common bile duct independently.[8,11,20]

Other irregularities of the gall bladder may be confused with the true duplicate condition. The "bilobed" gall bladder (vesica fellea divisa) is a partially subdivided gall bladder. This anomaly is the most common variation in the cat. There are two naturally occurring groups: (i) those in which the vesicle is slightly divided by a short cleft, and (ii) those in which the body of the organ is divided into two cavities by a longitudinal septum. Both groups exhibit asymmetrical development.[4,9] One cystic duct serves both compartments.

The "hour-glass" gall bladder is composed of two equal and functional cavities separated by a transverse septum; the proximal chamber empties into a distal one, which then empties into a single cystic duct. In the "Phrygian Cap" abnormality there is a much smaller proximal cavity separated from the distal compartment by a transverse septal constriction. Diverticuli of the gall bladder usually occur in the neck region of the gall bladder. These diverticuli are usually considerably smaller than the primary vesicle. When the diverticulum is large, the vesicle-diverticulum complex may resemble a bilobed gall bladder. The anatomically proved double gall bladders were observed in persons ranging from age 26 to 70.[7]

The incidence was equal in both sexes. "Double gall bladder" in males, age 14 and 16, was demonstrated by cholecystogram alone.[7]

The accessory gall bladder may vary greatly in size from the primary organ. Most often the vesicles of the "Y"-type gall bladder have been found in close proximity, occupying the same fossa. Usually they are invested in a common layer of connective tissue covered by a layer of peritoneum; thus making the initial anatomic diagnosis more difficult. The cystic ducts of the "Y"-type anomaly are usually bound together by fascia. The junction of these two ducts to form a common cystic duct may occur at different distances from its site of fusion with the common hepatic duct to form the common bile duct.

The bodies of the "H"-type anomaly are most often separated; occasionally they occupy different lobes of the liver: the accessory viscus may be situated under the left lobe of the liver, within the substance of the right lobe of the liver, on the undersurface of the liver, or in the gastrohepatic ligament. Either of the cystic ducts may join the hepatic or common bile duct at any point. Complete anatomical descriptions of the cystic ducts were available in 61 cases, of which 34 were completely duplicated ("H"-type), and 11 were "Y"-type gall bladders. Sixteen of these were classified as bilobed gall bladders. The arrangement of the ducts in 7 additional cases was not described.

The presence of double vesicles may be associated with independent function of each. Evidence of a disease process may be found in only one of the gall bladders.

Three triple gall bladders have been described.[9, 11, 16] Each vesicle also had its own cystic duct. Two of the gall bladders in each person were of "normal size," the third being somewhat smaller. Each report gave evidence of apparent function in all three gall bladders, although calculi were present in each viscus.

The embryology of the gall bladder has been studied in great detail.[4, 15] The vertebrate gall bladder makes its appearance at the 14 somite stage (3 mm.) of development (fourth week). A ventral pouch of foregut (entoderm) appears in the region of its junction with the yolk sac. This is the rudiment of the liver, gall bladder, bile ducts, and a portion of the pancreas. After the bud sacculates and differentiates into a caudal and larger craniad portion, the cranial division further divides into right and left halves which expand forward into the septum transversum to form the spongy network of cells that will become the liver. The caudal division of the primordium differentiates into the gall bladder and biliary tree by the 5 mm. crown rump embryo length (early in the second month). At this time the gall

bladder imbeds itself in hepatic tissue. Later it assumes a superficial position, possibly because the surrounding hepatic tissue atrophies secondary to mechanical pressure from the enlarging gall bladder.[6]

The gall bladder primordium grows craniad away from the duodenum and thus forms the future cystic hepatic and bile ducts by elongation. The lumens of the primitive gall bladder and bile ducts are occluded by proliferation of their epithelium so that they become solid cords during the second month of development, but by the seventh week they become patent again. There is a pronounced wrinkling of the gall bladder at that time. Occasionally the primordial solid bud subdivides, giving rise to a "bilobed" gall bladder with a common cystic duct, which is a common anomaly in the cat. It is likely that the septum of the bilobed gall bladder is a vestige of the "solid" stage of development.

There is still some question as to the derivation of the double gall bladder. It has been suggested that the anomaly originates either as a primary subdivision of the caudal primordium into two parts or as an outgrowth of a secondary vesicle from some other portion of the biliary tree subsequent to the formation of the definitive gall bladder. The evidence for the latter is more convincing. In man the bilobed condition is much less frequent than the duplex form. The simpler bilobed modification might be expected to occur more often than the complex double gall bladder, but such is not the case.

In the cat the accessory, ductular ("H"-type) gall bladder arises as an outgrowth of the hepatic or common ducts. A human embryo has been described with two diverticuli of the cystic duct which might have gone on to form a "Y"-type double gall bladder.[4]

Boyden's extensive studies on the embryology of the biliary system indicate that during the sixth week of vertebrate development a "vestibule" appears at the upper end of the embryonic choledochus into which all of the biliary and cystic ducts enter. From this "hepatic antrum" of the ductular system numerous outgrowths and vesicles commonly develop. In most circumstances these supernumerary buds begin to regress by the 15 mm. stage. Should one of them persist, an accessory gall bladder of the "ductular"-type would develop. At that time a diverticulum from the primary cystic duct might occur which could go on to develop into an accessory gall bladder of the "Y"-type. The "trabecular" gall bladder seems to originate from hepatic trabeculae bordering on the fossa vesica fellea. The cystic duct in this anomaly (found in 4 calves, 1 sheep, and 1 man[4] empties directly into one of the bile capillaries within the liver substance.

Abnormal valves of Heister and septa such as the "hour glass" and "Phrygian Cap" are most probably the result of vestiges of the solid stage of biliary tract development.[7]

REPORT OF A CASE

A double gall bladder of the "H"-type was found during the course of dissection of a 71-year-old, white, male cadaver.* The clinical history revealed no information concerning biliary tract symptomatology or disease. His demise was related to metastatic carcinoma of the prostate. No other anatomical anomalies were observed. The fundi of both gall bladders and the cystic ducts were located in the fossa vesica fellea and were invested in a common layer of connective tissue covered on the free surface by a layer of peritoneum. The initial examination did not suggest any anomaly other than an enlarged cystic duct. Sharp dissection of the closely adhered gallbladders and their ducts revealed a completely duplicate structure with two distinctly separate cystic ducts (see Fig. 1).

The right cystic duct measured 8.0 cm. from the neck of the gall bladder to the junction of the duct with the common bile duct. A 0.5 cm. stone was lodged in the midportion of the lumen of the right cystic duct. The left cystic duct was 5.0 cm. in length. The left cystic duct joined the right hepatic duct 3.2 cm. anterior to the junction of the right cystic duct with the common bile duct. The body of the right gall bladder measured 6 cm. (length) x 6 cm. (circumference). It contained two gallstones approximately 0.8 cm. in diameter. The left fundus was 6 cm. (length) x 6.5 cm. (circumference); no calculi were present.

Microscopic sections of the walls of both specimens showed the presence of mucosa and definite muscularis layers in each. Congealed bile was present in both gall bladders (characteristic of embalmed cadavers). Two cystic arteries arose from the right hepatic artery. The most superior of these vessels supplied the anterior-dorsal (hepatic) surfaces of both vesicles, while the inferior cystic artery was distributed over the ventral-posterior (free) surfaces of the gall bladders.

DISCUSSION

The preponderance of reports of double gall bladders by the surgeon and radiologist as compared with the few cases discovered at the dissecting table suggest that duplication of the gall bladder tends to be symptomatic. Clinical indications for cholecystography and/or surgery in addition to the high percentage of gallstones present in those cases which have been examined anatomically support the idea that double gall bladders are more susceptible to inflammation and lithiasis. Most often a pathological process was described in only one of the gall bladders, while the remaining viscus was apparently normal. Among 25 cases of duplicate gall bladders, the indefinite symptoms of pain, intolerance to foods, nausea, vomiting, and

* Cadaver #727.

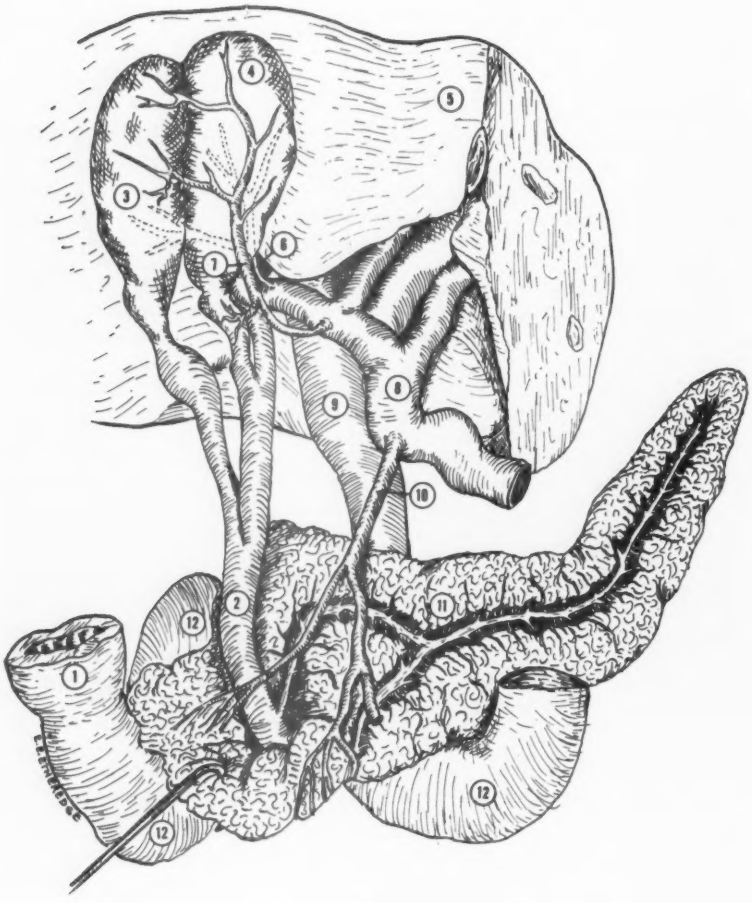

FIG. 1.

1. Pylorus
2. Common bile duct
3. Right gall bladder
4. Left gall bladder
5. Liver
6. Superior cystic artery
7. Inferior cystic artery
8. Common hepatic artery
9. Portal vein
10. Gastroduodenal artery
11. Pancreas (ducts exposed)
12. Duodenum

indigestion were present in 23.[7] Nine underwent surgery, at which time evidence for cholecystitis was found in all. There is, however, no evidence that the presence of the double gall bladder in the absence of disease gives rise to symptoms referable to biliary dysfunction. The presence of an accessory gall bladder has not been correlated with the occurrence of any other anatomical anomalies in the cases examined.

Cholecystography alone cannot be relied upon for the diagnosis of duplication of the gall bladder, since some reports have shown that x-ray findings were not confirmed upon operative examination. It is likely that some cases of double gall bladders are not visualized radiographically.

From the human cases reported there seems to be a striking tendency for variation in the direction towards double gall bladders of the ductular ("H") type: the ratio being 35:11.

SUMMARY

A double gall bladder of the "H" type was found during dissection of a cadaver of a 71-year-old male. The arterial supply of the biliary vesicles is described. Anatomical variations and the embryology of double gall bladders are discussed.

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R. YESNER*
R. D. SCHWARTZ**
H. M. SPIRO†

Pathology Service, Veterans Administration Hospital, West Haven, and the Department of Internal Medicine, Yale University School of Medicine

DUODENAL BIOPSY IN DIARRHEA AND STEATORRHEA‡

Beginning with Gee's[7] mention of atrophy of glandular crypts in 1888, many investigators of idiopathic sprue agreed that the significant changes in the small bowel at autopsy consisted of mucosal and villous atrophy, edema, and chronic mucosal inflammation and ulceration. Later, these observations were dismissed by Thaysen[18] as due to post-mortem autolysis. According to Suarez *et al.*,[13] Koppisch subsequently described shortened, clubbed, and blunted villi in 8 of 16 autopsied cases of sprue, and Adlersberg and Schein[1] found bizarre villous shapes or clubbing in 2 of 10 autopsied cases. It remained for small bowel biopsy to settle the question of post-mortem artefact, however.

Studies of the histological appearance of biopsy specimens of small intestinal mucosa have been concerned mainly with changes occurring in the jejunum in patients with sprue, both tropical and nontropical, and with celiac disease. The changes described range from blunting, mushrooming, and reduction in the size of villi to almost total flattening of the villous surface area.

This report presents observations of biopsy material which indicate that some mucosal changes in the duodenum are as striking and characteristic as those described in the jejunum, and that in severe chronic diarrhea there may occur quantitatively less extreme changes which are nonspecific in nature.

METHODS AND MATERIALS

Biopsy specimens were obtained by means of the small bowel biopsy tube devised by Shiner.[11] Twenty-two duodenal biopsies and three jejunal biopsies were obtained; one

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* Chief, Laboratory Service, VAH, West Haven, and Associate Clinical Professor Pathology, Yale University School of Medicine.
** Former Chief Resident, Medical Service, VAH, West Haven.
† Assistant Professor of Medicine, Yale University School of Medicine.
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to four specimens measuring approximately 3 x 4 mm. were obtained from each patient. In two cases of chronic steatorrhea, repeat biopsies were obtained at later intervals. In each case, mucosa, muscularis mucosae, and a small segment of submucosa were obtained. For comparison, duodenal specimens were obtained two hours' post-mortem from four patients with no known history of gastrointestinal disease. All photomicrographs were taken at x145 (Figs. 2, 4, 5, and 7) or at x170 (Figs. 1, 3, and 6) of sections cut perpendicular to surface.

RESULTS

Normal duodenum at autopsy. The autopsy specimens of normal duodenum were all quite similar. The villi were long, slender, and tended to lie against the mucosa. There was marked lysis and shrinkage of columnar and goblet cells which covered their surfaces. The glandular areas were much better preserved. Scanty mitoses were present in the neck area. A moderate number of lymphocytes, plasma cells, and eosinophils were present in the lamina propria. Brunner's glands were abundant and not dilated. Blood vessels were engorged, but no edema was present. As the jejunum was approached, both valves and villi became taller and there was better preservation of villous epithelium. Thickened or fused villi were rare.

The clinical pathology, biopsy site, and histopathology of the biopsy material are briefly documented in Table 1 for those cases without steatorrhea and in Table 2 for those cases with steatorrhea. In contrast to the post-mortem material, there was excellent preservation of structure, including the easily damaged surface epithelium.

Biopsies in cases without steatorrhea. Of the 17 cases without steatorrhea, the first 14 had predominantly slender, delicate, symmetrical, filiform villi, covered by columnar epithelium with a prominent cuticular membrane (Fig. 1). Of these 14 cases, the first 2 biopsies were jejunal and the last 12 were duodenal. Half the duodenal biopsies showed focal areas of villous hypoplasia and fusion (Fig. 1). Brunner's glands were occasionally mildly dilated (Fig. 2). In the remaining 3 duodenal biopsies of this group, atrophy, fusion, edema and flattening of villi occurred with a reduction in total thickness of the mucosa. These changes were mild in Case 15, consisting chiefly of hypoplasia, and marked in Case 16 (Fig. 3) and Case 17 (Fig. 4).

Biopsies in cases with steatorrhea. In Case 18, in which steatorrhea was present following subtotal gastrectomy, biopsy of efferent jejunum showed areas ranging from extreme atrophy of mucosa without flattening of the surface, to hyperplastic, edematous, clubbed villi. Of the remaining 5 cases in this group, 4 were considered to be cases of sprue on clinical, x-ray, and

TABLE 1. HISTOPATHOLOGY IN CASES WITHOUT STEATORRHEA

Case	*Sex*	*Age*	*Clinical diagnosis*	*Biopsy site*	*Histopathology*
1	M	62	Subtotal gastrectomy for ulcer; x-rays & gastroscopy negative; pulmonary tuberculosis	proximal jejunum	normal
2	M	52	Gastrectomy for gastric carcinoma; epigastric pain	proximal jejunum	normal
3	M	30	X-ray: antral gastritis	1st duodenum	focal hypoplasia of villi
4	M	50	Achylia gastrica; pernicious anemia	2d duodenum	normal
5	M	27	Severe diarrhea; x-rays: regional enteritis	2d duodenum	normal
6	M	34	Chronic relapsing pancreatitis	2d duodenum	focal hypoplasia and fusion, villi; incr. WBC
7	M	62	Achylia gastrica; intermittent dyspepsia; pulmonary emphysema	3d duodenum	normal
8	M	63	Achlorhydria; diarrhea; negative g.i. series	3d duodenum	normal
9	M	24	Bloody diarrhea; x-rays: ulcerative colitis	3d duodenum	normal
10	F	40	Severe diarrhea; scleroderma; multiple joint deformities; paresis of legs	3d duodenum	focal hypoplasia of villi
11	F	40	Diarrhea; anemia; normal g.i. series	3d duodenum	focal hypoplasia of villi
12	F	32	Diarrhea; anemia	3d duodenum	focal hypoplasia of villi
13	M	37	Diarrhea; abdominal pain; weight loss, 4 mos.	3d duodenum	focal hypoplasia of villi
14	M	62	Carcinoma of stomach; cirrhosis	3d duodenum	focal hypoplasia of villi; increased WBC
15	M	42	Rheumatoid arthritis; severe diarrhea	1st duodenum	small villi, thin mucosa
16	F	46	Chronic diarrhea	2d duodenum	atrophic villi, thin mucosa, irregular surface
17	M	60	1½ years diarrhea & weight loss; radioactive triolein, normal recovery	3d duodenum	atrophy of villi, edema thin mucosa

TABLE 2. HISTOPATHOLOGY IN CASES WITH STEATORRHEA

Case	*Sex*	*Age*	*Clinical diagnosis*	*Biopsy site*	*Histopathology*
18	F	50	Subtotal gastrectomy for ulcer; 4-6 fatty stools daily; anemia. X-rays: negative	efferent jejunum	focal hypoplasia and hyperplasia of villi
19–1	F	68	Celiac disease as a child. X-rays: malabsorption syndrome, sprue	2d duodenum	fused & flattened villi dilatation of Brunner's glands, marked
19–2	F	68	idem.	2d duodenum	idem.
20	F	47	Sprue	3d duodenum	fused & flattened villi, thin mucosa
21–1	M	46	Sprue	2d duodenum	fused & flattened villi, thin mucosa, dilated Brunner's glands
21–2	M	46	idem.	3d duodenum	villi & mucosa, idem.
22	M	32	Sprue; did well on gluten-free diet	3d duodenum	fused & flattened villi
23	M	45	Arthralgia. X-ray: abnormal small bowel pattern; low xylose tolerance	2d & 3d duodenum	Whipple's disease

laboratory evidence, and are duodenal biopsies. In Case 19 in which steatorrhea had been present since childhood in a 68-year-old woman, the villi were flattened and fused to form an even surface, without reduction of the total thickness of the mucosa. There was an increase in leukocytic infiltration and Brunner's glands were markedly dilated. Repeat biopsy at a later date showed the same type of flattened mucosa (Fig. 5). In Cases 20 and 21, the villi were broadened, flattened, and fused, with an over-all reduction in mucosal thickness (Fig. 6). These changes were again demonstrated by repeat biopsy in Case 21. Biopsy in Case 22, as in Case 19, showed a normally thick, chronically inflamed mucosa with a histological pattern suggesting that the flattening of the surface was due to broadening and fusion of the villi rather than to simple atrophy (Fig. 7).

The last case of steatorrhea was proved histologically to be Whipple's disease with markedly hyperplastic and clubbed villi, macrophages stuffed with PAS.-positive material, and dilated lymphatics. The subjacent Brunner's glands were markedly dilated (Fig. 8).

Some degree of congestion and edema was present in practically all specimens. Variable numbers of lymphocytes, plasma cells, and eosinophils were present in the lamina propria, chiefly between the glands in the region of the muscularis mucosae. Normal numbers of goblet cells and glandular mitoses were always present. The superficial columnar cells tended to be slightly to moderately shorter whenever the villi were flattened, in both sprue (Fig. 6) and nonsprue (Fig. 8). No vacuolization was noted in these cells, nor could abnormalities of the Paneth or argentaffin cells be detected. No abnormalities of the muscularis mucosae were present, nor was there any evidence of submucosal infiltration. Focal dilatation of Brunner's glands was noted in the 4 cases of sprue (Fig. 5), the case of Whipple's Disease (Fig. 8); and to a lesser extent in a case of chronic diarrhea without steatorrhea but with marked atrophy of villi (Fig. 3), and in a case of chronic relapsing pancreatitis (Fig. 2).

DISCUSSION

Normal jejunum. Butterworth and Perez-Santiago[5] reported that in 15 normal jejunal biopsies taken at laparotomy, thickened or "double" villi did not exceed one per low power field of the microscope. Descriptions of normal jejunum by other investigators[6, 8] using a jejunal biopsy tube, are similar. Villi are long and slender, lymphocytes, plasma cells, eosinophils, and macrophages are always abundant in the stroma. Gastrectomy does not always disturb this pattern. Baird and Dodge[8] described 11 jejunal biopsies taken at gastrectomy for peptic ulcer. The villi occupied

two-thirds of the mucosal thickness. Our two biopsies of postgastrectomy nonsteatorrhea cases produced specimens like those described and may be considered entirely normal.

Normal duodenum. Our autopsy specimens of normal duodenum resembled normal jejunum, except for Brunner's glands and somewhat shorter villi. A considerably greater variation in normal duodenal pattern was described by Doniach and Shiner[8] in 25 patients. The specimens, which were removed by biopsy tube, were described as showing marked variation in thickness of mucosa and intensity of cell infiltration, and also variability in shape of villi, which were occasionally branched, plumper and more club-shaped than those in the jejunum. They noted numerous mitoses in the mid-portion of the glands.

Duodenal biopsies in gastrointestinal disease without steatorrhea. Doniach and Shiner have reported three patients in this category with abnormal duodenal mucosa. One was a case of gastric ulcer with thin, irregular, branching villi and thin mucosa. A second was a case of duodenal ulcer with irregular villi which were mostly short and blunted, with thin mucosa and distended glands. The third was a case of iron-deficiency anemia with moderately irregular villi. Since these authors have described marked variation in the thickness of normal duodenal mucosa, it is difficult to know whether these biopsies fell within normal range. At any rate, none apparently showed severe atrophy or suggested sprue.

Six of our duodenal biopsies from a variety of conditions, including pernicious anemia, regional enteritis, achylia gastrica, achlorhydria, and gastric carcinoma, resembled our autopsy specimens of duodenum, except that they were far better preserved, showing slender villi, normal Brunner's glands, and a uniform mucosal pattern. An additional six duodenal biopsies from cases of antral gastritis, chronic relapsing pancreatitis (Fig. 2), scleroderma (Fig. 1), and three cases of diarrhea showed only focal villous hypoplasia, probably within normal range. However, three duodenal biopsies from cases of severe diarrhea showed villous hypoplasia and atrophy of a more general and extreme type (Figs. 3, 4). In each case, the mucosa was hypoplastic or atrophic with reduction in over-all thickness, but without a flattening of the surface. It it apparent that severe chronic diarrhea without steatorrhea may be associated with atrophic changes in the duodenal mucosa. In all duodenal biopsies congestion was regularly present. Since the specimens were obtained in the early morning, prior to breakfast, it is unlikely that this is due to digestion and absorption, as Baird and Dodge[3] assumed in four of their postgastrectomy jejunal biopsies.

Jejunal biopsies in postgastrectomy steatorrhea. Similar atrophic changes were found focally in the jejunum in the case of postgastrectomy steatorrhea. This is consistent with the findings of Paulley,[8] who in 1957 described a case of post-gastrectomy steatorrhea in which biopsy was normal, but at autopsy patchy atrophy was present. Of Baird and Dodge's[2] five cases of postgastrectomy steatorrhea, only one presented villous atrophy, again suggesting the presence of only focal changes.

Jejunal biopsies in sprue. The biopsy specimens of sprue in the literature deal almost exclusively with the jejunum. Paulley[9] in 1954 reported broadened and clubbed villi and marked infiltration by plasma cells and eosinophils in two jejunal biopsies in patients with idiopathic steatorrhea. Butterworth and Perez-Santiago[5] obtained jejunal biopsies from six patients with sprue and proved malabsorption, generally finding edema, inflammation, and changes in the number and contour of villi. Shiner[10] in 1957, describing histological changes in sprue in specimens taken by a jejunal biopsy tube, noted that they occurred in some cases but not all, that they consisted chiefly of villous atrophy, which had otherwise been observed only in one case of pernicious anemia, and that in the severest cases a uniform flattening occurred with almost total obliteration of the villi. She further noted the same changes occurring after treatment and remission in patients controlled on steroid therapy. Bolt *et al.*[3] in 1958 reported biopsies taken with a jejunal biopsy tube from seven patients with sprue. The specimens were all similar, differing distinctly from normal small bowel. There was flattening of the epithelial surface of the villi, marked cellular infiltration of the tunica propria, coalescence of villi, and a corkscrew arrangement of the glands. They considered these changes diagnostic of the disease, treated or untreated.

Duodenal biopsies in sprue. Doniach and Shiner reported gross blunting of villi and great loss of villous surface on biopsy of a case of steatorrhea with megaloblastic anemia.

In the four cases of sprue in which we have examined duodenal biopsy material, the mucosa was markedly and generally abnormal (Figs. 5, 6). The chief abnormality consisted of a widening and fusion of the villi to produce a flat mucosal surface, without necessarily involving a reduction in over-all thickness. The loss of villous pattern was to make the specimens generally unrecognizable as small bowel. These changes were found only in sprue, and in all the cases of sprue. As in the jejunal biopsies of Shiner[10] and Bolt *et al.*[3] there was no histological recovery with treatment and remission in two cases rebiopsied. It seems apparent that in idiopathic steatorrhea, or sprue, the histological changes in the duodenum and in the jejunum

are identical, consisting essentially of broadening and fusion of the villi, resulting in a flattened mucosa of greatly diminished surface. In the duodenum this ironing out of the mucosa may or may not be associated with over-all reduction in mucosal thickness. There is no cork-screwing of the glands suggested in the jejunal biopsy material of Bolt *et al.*[3] The appearance of flattened, coalesced villi seems to be characteristic of sprue in the duodenum as well as in the jejunum. Atrophic duodenal mucosa with blunted villi is not characteristic of sprue, but occurs in other chronic diarrhea.

Duodenal biopsy in Whipple's disease. The one entity in which completely specific histological confirmation of a clinical diagnosis is possible is Whipple's disease. A case of Whipple's disease was diagnosed by Bolt *et al.* by jejunal-tube biopsy. The case here presented documents involvement as proximal as the first duodenum in this disease.[4] Brunner's glands are markedly dilated, as they were in sprue (Fig. 8).

CONCLUSIONS

In the duodenum of patients with severe chronic diarrhea, the villi may be normal, slender structures or may show foci of hypoplasia. In a smaller number of these patients, there may be marked atrophy of villi with an increase in cellular infiltration and thinning of mucosa. Associated with these changes, Brunner's glands may be dilated. In severe chronic sprue, the duodenal villi are broadened and fused, so that the mucosal surface is markedly flattened although the entire thickness of the mucosa may not be lessened. These changes are accompanied by increased numbers of plasma cells and eosinophils, along with dilatation of Brunner's glands. They were present in all four cases of sprue, both before and after treatment, and are regarded as characteristic. Whipple's disease is a variety of secondary steatorrhea, the pathognomonic lesions of which may be found as high as the proximal duodenum.

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FIG. 1. Case 10: chronic diarrhea and scleroderma. Third duodenum: normal villi with occasional small or fused variant, x170.

FIG. 2. Case 6: chronic relapsing pancreatitis. Second duodenum: normal villi, mildly dilated Brunner's glands with periglandular cellular exudate, x145.

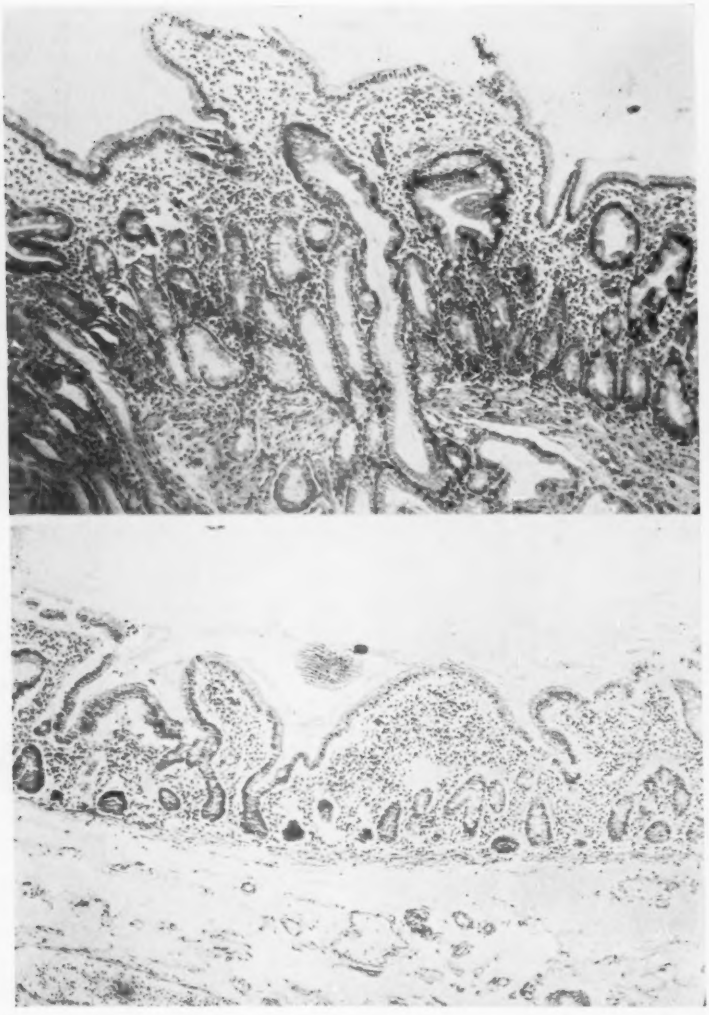

FIG. 3. Case 16: chronic diarrhea. Second duodenum: atrophic fused villi with irregular surface, mildly dilated Brunner's glands, x170.

FIG. 4. Case 17: chronic diarrhea. Third duodenum: atrophic mucosa, irregular surface, x145.

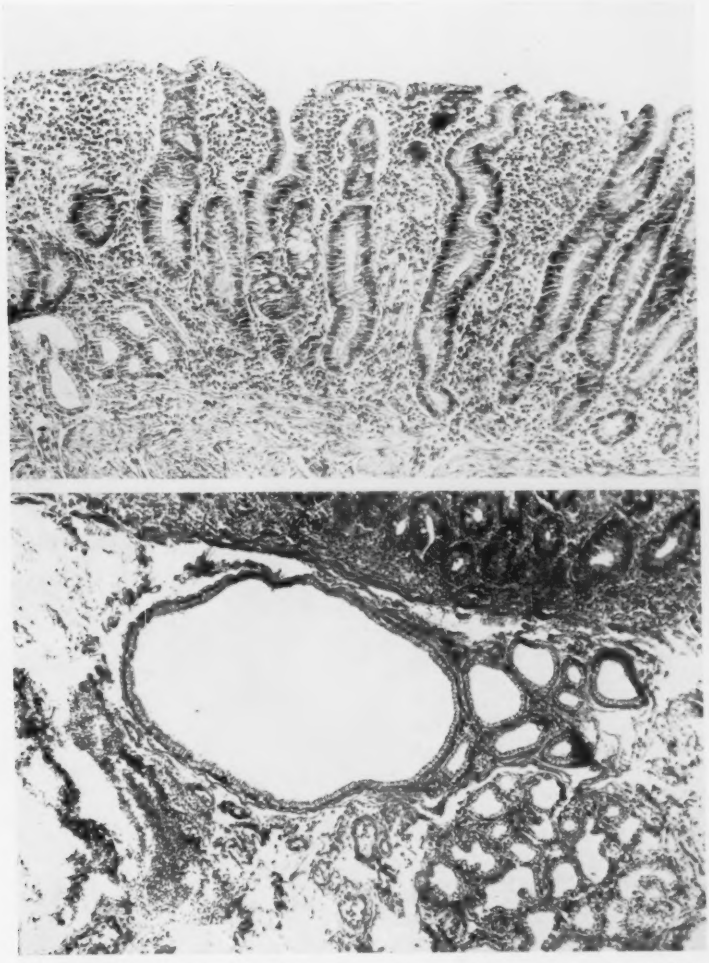

FIG. 5. Case 19: sprue. Second duodenum. Top: widened and fused villi. Below: marked dilatation of Brunner's glands, x145.

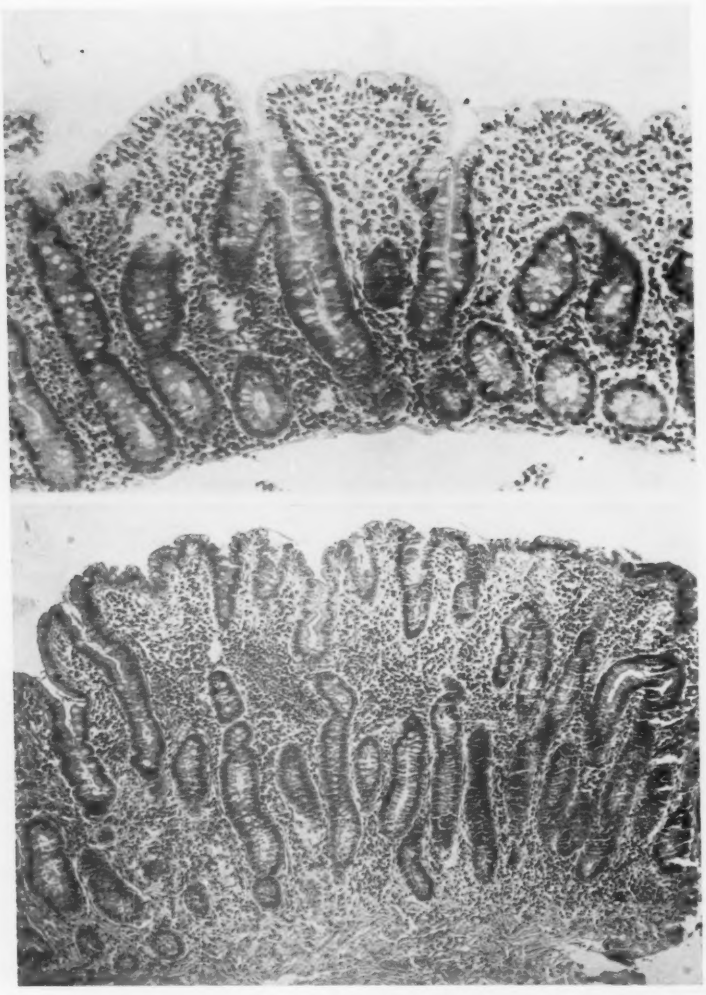

FIG. 6. Case 20: sprue. Third duodenum: widened and fused villi with flattened surface, thin mucosa, x170.

FIG. 7. Case 22: sprue. Third duodenum: fused villi, flattened surface, normally thick mucosa, x145.

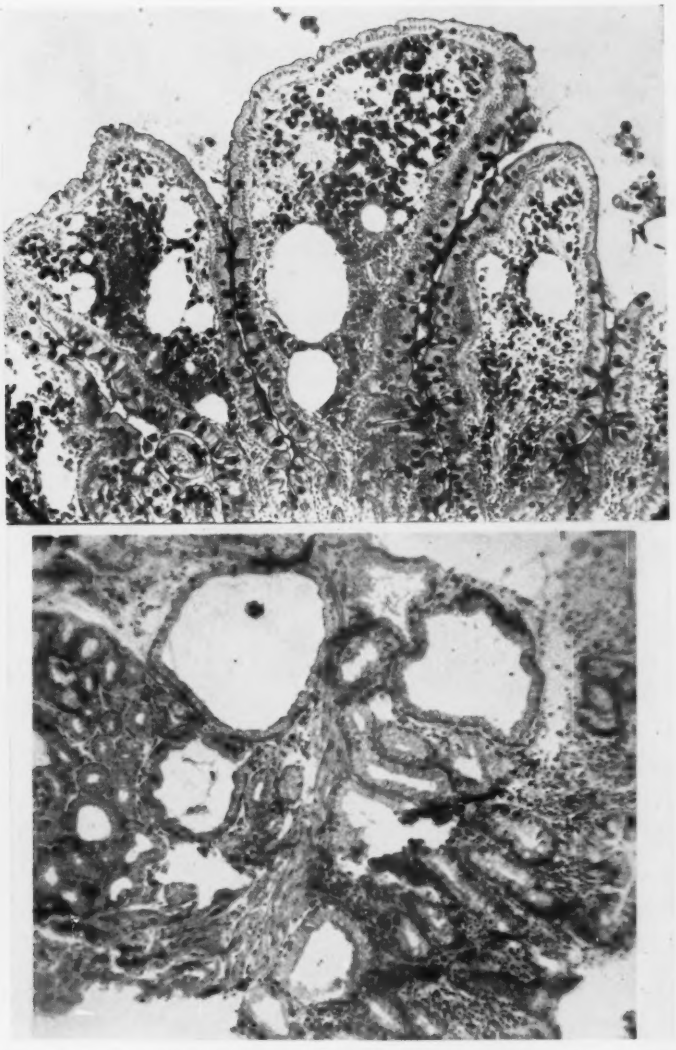

FIG. 8. Case 23: Whipple's disease. Second duodenum. Top: markedly enlarged, clubbed villi with PAS-positive macrophages and dilated mucosal lymphatics. Below: markedly dilated Brunner's glands, x145.

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MASAYOSHI YAMAGUCHI*

POPULATION TREND AND PUBLIC HEALTH IN JAPAN**

The "population problem" is the most fundamental of the problems which confront the world today. In recently developing countries, the mortality rate was lowered after the war owing to the marked improvement of public health. The fertility rate remained uncontrolled at an extremely high level in these same countries. Furthermore, even in countries which now have high mortality, public health activities will be strengthened by their own efforts and with the help of international organizations, in conformity with the principle of the World Health Organization; and the death rate will surely be lowered in the future.

It is feared the explosive increase of population, predicted in these countries for the near future, will hinder their modernization. Delayed modernization, in turn, is considered as an obstruction to the adjustment of population increase, giving rise to the great possibility of a vicious circle between them.

Basic questions regarding the world's population problem are how to regulate the population increase rate and how to improve the economical condition of the country to adjust the increased population, and by doing so, how to raise the living standards of the people. When discussing the population problem, therefore, economical and social factors usually come to the front. At the same time, however, we have to keep in mind the fact that as the population trend is a function of births and deaths, it naturally involves numerous important factors which deal with the public health. By reducing the death rate, by regulating the fertility rate and by adjusting the increased population, we have to deal always with the problems of public health.

Japan was at first a country with high fertility and high mortality, then with high fertility and moderate mortality; now she is a country with low fertility and low mortality. Before explaining the rapid postwar population changes, attention will be given to the way the population changed

* Director, National Institute of Industrial Health, Japan; Chairman, Board of Directors, Japan Anti-Tuberculosis Association; Director of Public Health, Japan (retired).

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during the 80 years beginning in the late 19th century as a result of the modernization of Japan.

In 1872, not long after the Meiji Restoration, the new government of Japan carried out a census of the population with the object of establishing a modern national registration system. The result showed that the population was approximately 35 million. At that time the government was making great efforts to modernize Japan, but the years before the beginning of the 20th century can be called the era of preparation. Modernization began to develop rapidly once this century had begun. As it progressed, the rate of increase of the population also began to climb gradually. In 1911, the population was 50 million; by 1925 it had increased to 60 million; and in 1936 it was 70 million. The population of Japan had thus doubled during the 64 years from 1872 to 1936.

At the most recent census, taken in 1955, the population of Japan was 89.3 million. Calculations of subsequent births and deaths, together with emigration and immigration figures, bring this year's population as of January 1, 1959 to an estimated 92 million and over. Compared with 46 million, which was the population of Japan in 1903, it took only 55 years to become double. In other words, the rate of increase of the population became higher than before.

However, in the ten years after World War II, due to the marked decrease of the birth rate, the rate of increase of the population became very slow. Japan has now a population of over 92 million, which is the fifth largest population in the world, after China, India, Soviet Russia, and the United States. She lost 46 per cent of her former territories as a result of World War II, and this great population is living in an area of about 370,000 square kilometers which is equivalent to that of the state of California. Consequently, the population density is very great with about 249 people living in each square kilometer. This population density is third only to Holland and Belgium. But, topographical conditions in Japan must also be considered. In Holland and Belgium, two-thirds of the land can be used for cultivation; in Japan, with its many mountains, the proportion is one-sixth.

With this change of the population and its extremely high density in a small country, Japan has faced and overcome many problems relating to public health, some of which will be presented.

From 1900 to about 1925, the death rate of Japan was around 20 every year, except for the high rates in 1918 and 1920, because of the world-wide epidemic of influenza, of 27.3 and 25.4, respectively. It began to decline in 1926 and decreased in the following years until it went below 16 in 1941

despite the war. After the war it decreased to 11.9 in 1948 in spite of the fact that the considerable incidence of deaths must have occurred during and directly after the war period. It further continued to decrease to as low as to 7.4 in 1958.

By analysing the annual change of death rate by leading causes, it is clear that the improvement of the mortality rate in Japan is attributable principally to the marked decrease from communicable diseases such as acute enteritis, tuberculosis, pneumonia, and so forth. The death rates from these communicable diseases were around 200 or more per 100,000 population about 20 years ago, but they have dropped down to 25.1, 39.3, and 49.7, respectively, in 1958.

From the viewpoint of age groups, the improvement of death rates is conspicuous in the younger generations. For example, expressing the death rates by index and taking that of 1953 as 100, the death rate of all age groups in 1957 was 49.2, whereas those of the age groups between 10 and 14, between 15 and 19, between 20 and 24 were 21.2, 16.0, and 23.3 respectively.

The most remarkable decline of the death rate in the younger age groups occurred in tuberculosis, especially during the past 15 years. These figures will be presented in some detail. Until recent years, namely until 1950, tuberculosis had been the leading cause of death as recorded in the vital statistics in Japan. From 1900 until 1923, the death rate due to tuberculosis was 200 or more per 100,000 population every year. From 1924 to 1935, the figures were slightly below 200. However, after 1936 it began to increase, possibly due to World War II, reaching 235.3 per 100,000 population in 1943. After the war, since 1947, it showed a gradual tendency to decrease for several years; then since 1951 the curve of decline became very sharp. In 1958, it became as low as 39.3 per 100,000 population which is only one-sixth as compared with the figures of 1943.

In rank as a leading cause of deaths, tuberculosis became second in 1951, giving the top position to cerebral hemorrhage. In 1958 it dropped to sixth after cerebral hemorrhage, cancer, senility, heart disease, and pneumonia including bronchitis. Turning to the tuberculosis death rate by age groups, the most remarkable decline has occurred in the age group between 15 and 19, and in the age group between 20 and 24. Until recently, the special feature of the tuberculosis death rate in Japan was the extremely high peak in the younger generations, especially in the age groups between 15 and 29. For instance in 1943, the tuberculosis death rates for age groups between 15 and 19, between 20 and 24, between 25 and 29 were 402.8, 617.4, and 514.5 respectively, whereas that of all age groups was 235.3, as just men-

tioned above, and the death rates by age groups were less and less as the age advanced.

Since 1947, the high peak in younger generations became lower and lower, and at the same time, the highest rates shifted to older age groups. For instance, in 1958, the death rates for the age group between 15 and 19, between 20 and 24, between 25 and 29 were 7.4, 20.1, and 38.0 respectively, and they are surprisingly low as compared with the figures for 1943, mentioned before.

Thus, although the absolute value of the tuberculosis death rate in Japan is still comparatively high as compared with the figures in the United States, Canada, Denmark, and so forth, the shape of the curve of the death rate by age has become very similar to those of the above mentioned countries.

Although there may be many factors causing these decreases of death rates, especially those of communicable diseases, we are confident that, as illustrated in tuberculosis, they are mainly because of the progress of medical science and public health. The progress of medical science has facilitated the discovery of new insecticides, new vaccines, and various antibiotics which are invaluable as effective weapons for prevention and treatment of disease. Improvement in the public health program carried out vigorously by the health center activities with the cooperation of community effort has successfully utilized these weapons for the prevention, the early detection, and the early treatment of the diseases and to decrease the death rates due to these diseases.

In public health activities there are two categories, one of which is carried by means of guidance of the public through health education and the other is performed by using the police power. Until the latter part of the Second World War period, even after the present health center system was set up in 1937, the public health activities in Japan were carried out, administratively, chiefly by using police power. Most of the public health administration was one of the functions of the police department at the local level, and the attitude of the public was rather passive. This is not public health activity in its true sense, and the effectiveness of health work in these circumstances is limited.

After the war, however, all activities relating to public health were centralized in the health center at the local level, and since then the police department has had no concern with public health administration. More emphasis has been paid to the health education of the public, especially to stimulate the community health organizations to initiate their own activities. By these efforts, public health activities initiating from small com-

munity organizations have become more numerous and have met with success in various aspects of health work, such as maternal and child health work, insect and rodent control activities, family planning programs, and so forth.

As a result of the improvement of death rates, especially in younger age groups, the life-expectation of the Japanese people has become 20 years longer within the 20 years; in 1958 the figures for male and female were 65.0 and 69.6 years respectively. If one considers the life-span of women, however, which is over 70 years in more than ten of the civilized nations of the world, it is clear that the mortality rate in Japan has not yet come down to the satisfactory level. Particularly, we have to take into account the fact that the population of Japan is relatively young and the proportion of the aged with high possibility of deaths is yet relatively small, and crude death rates may increase in the future.

With a definite tendency towards an aging population, more effort should be made in public health activities to attack the diseases causing the deaths of older age groups, which is the common object of the civilized countries in the world.

The birth rate is another factor in population trends. Until recently, Japan was a country with unusually high fertility rates. From 1900 to 1945, namely, until the end of World War II, the birth rates in Japan were between 30 and 35 per 1,000 population. With the baby boom following on the heels of the postwar marriage boom, Japan's birth rate in the three years 1947 to 1949 shot up above prewar levels, reaching 34.3 per 1,000 population in 1947. Since 1950, however, the curve has been going down rapidly at a rate rarely observed before. The birth rate in 1958 was 17.9 per 1,000 population and could probably be rated among the lowest in the civilized nations.

In most of the civilized countries which now have low fertility rates the decline of the birth rate to the present level took place in 30 years or more, whereas in Japan it has occurred in less than 10 years. The reason for this sudden drop in the birth rate can be found in the impoverished conditions prevailing directly after the war and to the unavoidable endeavours made to keep up living standards. The method used to lower the birth rate was at first, unfortunately in most cases, abortion, since the knowledge of contraception was limited to members of particular social strata in urban areas.

In 1949, with the aim of preventing unnecessary black-market induced abortion, the Eugenic Protection Law was amended with an article to legalize abortion. According to this article, a pregnant woman could have an abortion operation by an authorized doctor designated by the local med-

ical association if she had a proper reason from the eugenic point of view, or if she was considered to be so unhealthy that the continuation of her pregnancy or the delivery would be harmful. In that case, the doctor was obliged to get the confirmation of another authorized doctor and the permission of the Local Eugenic Protection Committee established in the health center. This last requirement was deleted late in 1952.

This amendment of the Eugenic Protection Law has accelerated the practice of abortion and the number of reported cases has increased year by year. The number of reported cases of legal abortion in 1949, 1950, and 1951 was 246,104, 489,111, and 638,350 respectively. The abortion operations, even if they are properly done, sometimes have ill effects on the health of mothers. According to the studies made by the Japan Gynecology Society analysing the results of about 39,550 cases of abortion in 1924, deaths directly related to the operation occurred in about 0.2 per cent and relatively serious ill effects appeared in about 2.6 per cent of the cases. Therefore, in 1951 the Japanese Government, trying to prevent abortion in the interest of mothers concerned, decided on a policy of propagating knowledge of contraception. Since then it has been carried out actively and extensively as a public health program giving knowledge about contraception individually and in groups and giving guidance on how to plan a family through health centers scattered all over the country.

The principle of propagating contraception was as follows:

1. To educate the people concerning the ill effects of unnecessary induced abortion and the meaning of family planning, utilizing such media as pamphlets, leaflets, talks, slides and films.
2. To educate the mothers concerned through group discussion in smaller groups utilizing the opportunities of mothers' classes and other similar gatherings.
3. To educate the mothers concerned by giving individual guidance in the use of contraceptives. This was given by doctors and by authorized midwives and nurses who have received special training for this purpose.

Within three years, consultation clinics were set up in every health center providing materials for education, and many of the midwives received special training for individual guidance and joined this movement. The government appropriated the money in its budget to give necessary contraceptives free to the poor people who could not afford to purchase them and also appropriated the money to pay midwives for consultation work. With the guidance of health centers or voluntary organizations, a large number of small community organizations were established as model districts for this movement throughout the country.

A number of relatively large industries were interested in this movement and recognized its benefits for the welfare of the families of their workers and promoted this family planning program as a policy of their enterprises. In 1954, with the recommendation of the Population Problems Inquiry Council, the government decided to spread the practice of family planning not, as hitherto, with the idea of protecting the mothers only, but as an integral part of its population policy.

As the result of such work, the number of abortions, which reached a peak in 1955, with the figure reported as 1,179,143, seems to be showing a gradual tendency to decrease, and the knowledge of family planning is spreading. According to the data from the Public Opinion Survey by the Population Problems Research Council of the Mainichi Newspapers, the figures of proportion of couples practising contraception in 1950, 1952, 1955, 1957, and 1959 are 20 per cent, 26 per cent, 34 per cent, and 42.5 per cent respectively.

Although it is extremely difficult to estimate the effect of contraception on the prevention of pregnancy, it is assumed that the ratio between contraception and abortion for suppression of births was, roughly speaking, about the same in 1958. Abortion should be replaced by contraception to maintain the present level of fertility rate.

The Institute of Population Problems of the Ministry of Health and Welfare has made an estimate of Japan's population in 1965, based on the figures obtained in the 1955 census, and analysing the trend in birth and mortality rates. They have further extended their estimate, assuming that the fertility rate of women at any given age and the mortality rate according to sex and age as estimated for 1965 would remain constant. According to this assumption, the population of Japan in 1965 will be 96.4 million, that of 1970 will be 99.6 million, and the population in 1975 will be 102.7 million.

Even more important than the size of the population is the rapid change in its age distribution caused by the postwar drop in the mortality rate and, in particular, by the decrease in the birth rate. In the ten years between 1955 and 1965, Japan's total population will increase by about 7 million, but the increase in the productive population—between the age of 15 and 64—will be as much as 13 million and the population of people over 64 will increase by 1.4 million. Compared to this, the population of children under the age of 15 will decrease by 7 million.

How can we provide employment for this huge productive age population in the coming years and, to do so, how can we raise the rate of economic growth and maintain it at a high level? With the expansion of the pro-

ductive age population from the present time to the near future, the number of unemployed may increase further, if the present situation does not improve. The government is working hard in preparation for this crisis.

This fact brings forward again an important and serious problem to the public health of Japan. According to the national health survey carried out by the Ministry of Health and Welfare, the sickness rate of the household which receives public assistance was 19.8 per cent as on April 15, 1958, whereas that of the general household was 3.4 per cent. The same tendency is clearly shown in the tuberculosis prevalence survey and mental health survey carried out by the Ministry in recent years. Poverty and disease constitute a vicious circle.

Japan may be considered to have coped successfully with its serious population problems in that the population increase rate has been brought down notably by the quick control of its fertility rate. However, despite the decrease of births that has taken place during a surprisingly short period, the productive age population will increase by an extraordinarily high rate ten years from now, resulting in serious problems in connection with employment and the public health.

With the present policy of expanding the labor market and raising the economic standard of the people, public health service should be strengthened. Increased effort is necessary to alleviate the poor economic conditions among the unemployed and to diminish morbidity for the welfare of our people and mankind in general.

DANIEL L. GILBERT*

Department of Physiology, Albany Medical College of Union University, Albany, New York

BUFFERING OF BLOOD PLASMA**

Buffering of the blood plasma in man depends upon the red blood cells, chemical buffers of the blood and tissue cells, respiratory system, and the kidneys. It is the purpose of this paper to give some quantitative estimates of the role of these factors in maintaining the pH of blood plasma.

Addition of nonvolatile acids or alkalies. Within the physiological range, the changes in the bicarbonate concentration of true plasma (plasma in contact with red blood cells) as caused by altering the pCO_2 appears to be proportional to the pH change[2] and can be expressed as:

$$HCO_3^- = -a\,pH + b \qquad (1)$$

where a and b are constants. If a nonvolatile acid is added, it is possible to maintain the same pH by reducing the pCO_2. When this occurs, all of the added acid (if it is completely dissociated) is buffered by the HCO_3^- system and the reaction can be written as:

$$H^+ + HCO_3^- \rightarrow H_2CO_3 \qquad (2)$$

As used here, the symbol H_2CO_3 refers to the sum of the hydrated and nonhydrated forms of physically dissolved carbon dioxide. Other buffer systems have no net participation. This can be shown by one form of the Henderson-Hasselbalch equation:

$$pH = pK' + \log \frac{\text{Total conc.—Acid conc.}}{\text{Acid conc.}} \qquad (3)$$

where pK′ is the pK′ of the weak acid, Acid conc. is the concentration of the weak acid, and Total conc. is the concentration of the anion of the weak acid not only in the free dissociated form but also in the undissociated form as the acid. This equation contains the three variables, pH, Total conc., and Acid conc. If the Total conc. remains the same, and if the pH is held constant by adjustments of pCO_2, then the Acid conc. is also not changed and there is no net participation of the buffer system. The millimoles of HCO_3^- must

* Assistant Professor of Physiology.

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therefore be reduced by the milliequivalents of acid added. The new relationship between the HCO_3^- concentration and pH can be represented as:

$$HCO_3^- = -a\,pH + b - A \tag{4}$$

where A is the concentration (in mEq/L) of the added acid (assuming the acid is completely dissociated). The concentration of the acid should be in terms of mEq/L blood water. (The Donnan equilibrium causes a slight unequal distribution of diffusible ions across the red cell membrane and for this purpose can be neglected.) Blood is composed of approximately equal volumes of red cells and plasma.[8] Therefore the amount of protein in the blood is equal to the average of the amount of protein in the red cells and in plasma. Since the protein in the red cells and plasma is respectively 35 per cent[9] and 6 per cent,[8] the protein or solid phase in the blood is 20 per cent. The remainder or 80 per cent of the blood is essentially water and therefore there are 0.8 liters of blood water per liter of blood. The buffer value "β"[m] can then be obtained by differentiating equation (4) with respect to pH:

$$\beta = -\frac{d\,A}{d\,pH} = a + \frac{d\,HCO_3^-}{d\,pH} \tag{5}$$

The value of $\frac{d\,HCO_3^-}{d\,pH}$ can be obtained by the use of the Henderson-Hasselbalch equation:

$$pH = pK' + \log\frac{HCO_3^-}{H_2CO_3} = pK' + \frac{1}{2.3}\ln\frac{HCO_3^-}{Total\ CO_2 - HCO_3^-} \tag{6}$$

where the Total $CO_2 = H_2CO_3 + HCO_3^-$. By substitution, the following are obtained:

$$\beta = a + \frac{2.3\,(H_2CO_3)\,(HCO_3^-)}{Total\ CO_2} + \frac{HCO_3^-}{Total\ CO_2}\frac{d\ Total\ CO_2}{d\,pH} \tag{7}$$

$$\beta = a + 2.3\,(HCO_3^-) + \frac{HCO_3^-}{H_2CO_3}\frac{d\,H_2CO_3}{d\,pH} \tag{8}$$

If the system is not in contact with a gas phase, then the Total CO_2 does not change, and equation (7) becomes:

$$\beta_{tc} = a + \frac{2.3\,(H_2CO_3)\,(HCO_3^-)}{Total\ CO_2} \tag{9}$$

where β_{tc} is the buffer value due to the total chemical buffers. For a bicarbonate solution, the value of "a" is equal to zero within the physiological range and so:

$$\beta_b = \frac{2.3\ (HCO_3^-)\,(H_2CO_3)}{\text{Total } CO_2} \tag{10}$$

where β_b is the buffer value due to bicarbonate. The value of "a" therefore represents the buffer value of the chemical buffers excluding HCO_3^- and so $a = \beta_c$ = buffer value of chemical buffers excluding bicarbonate.

For a phosphate solution, it can be similarly shown by differentiating the Henderson-Hasselbalch equation that the buffer value is:

$$\beta_p = \frac{2.3\ (H_2PO_4^-)\,(HPO_4^=)}{H_2PO_4^- + HPO_4^=} \tag{11}$$

where β_p is the buffer value due to phosphate.

If the system is equilibrated in contact with a gas phase, then the value of $\frac{\text{d Total } CO_2}{\text{d pH}}$ will depend upon the pCO_2, since H_2CO_3 is proportional to the pCO_2 (Henry's Law). The buffer value due to the presence of a gas phase, β_g, can be obtained by subtracting equation (9) from equation (7):

$$\beta_g = \frac{HCO_3^-}{\text{Total } CO_2} \frac{\text{d Total } CO_2}{\text{d pH}} \tag{12}$$

Thus, the total buffer value is equal to the sum of the individual components:

$$\beta = \beta_c + \beta_b + \beta_g = \beta_{tc} + \beta_g \tag{13}$$

For average arterial true plasma,[3] a = 21.6 mM/pH, HCO_3^- = 24.0 mM, H_2CO_3 = 1.2 mM, Total CO_2 = 25.2 mM, pH = 7.40, and pCO_2 = 40 mm Hg. The value of β_c is therefore equal to 21.6 mEq/L-pH (mEq/L per pH unit). For separated arterial plasma (plasma not in contact with red cells), a = 5.4 mM/pH[2], and so the value of β_c for the plasma is 5.4 mEq/L-pH, and the remainder, 16.2 mEq/L-pH, is the β_c for the red cells. It can be estimated from normal plasma ranges[3] that $H_2PO_4^-$ = 0.24 mM and $HPO_4^=$ = 0.96 mM, and so β_p only amounts to 0.4 mEq/L-pH. The buffer value of plasma protein is determined by difference and amounts to 5.0 mEq/L-pH. The value of β_b is only 2.6 mEq/L-pH. These values of β_c and β_b agree with those originally obtained by Van Slyke,[21] and point out that most of the chemical buffering in whole blood is due to the red blood cells.[6]

If the system is in contact with a gas phase of infinite volume, then the value of H_2CO_3 must be constant according to Henry's Law at equilibrium and by definition, β_{gA} = buffer value due to a gas phase at a constant pCO_2. Another buffer value, β_{gR} can be defined as:

$$\beta_{gR} = \beta_g - \beta_{gA} = \beta - \beta_A \tag{14}$$

where β_A is the total buffer value of the system at a constant pCO_2. Since $\frac{d\ H_2CO_3}{d\ pH}$ is zero under this condition, equation (8) reduces to:

$$\beta_A = a + 2.3\ (HCO_3^-) \tag{15}$$

Substitution of equation (7), equation (12), and equation (15) into equation (14) leads to:

$$\beta_{gA} = 2.3\ \frac{(HCO_3^-)^2}{\text{Total } CO_2} \tag{16}$$

Similarly, substitution of equation (8) and equation (15) into equation (14) results in:

$$\beta_{gR} = \frac{HCO_3^-}{H_2CO_3}\ \frac{d\ H_2CO_3}{d\ pH} \tag{17}$$

For arterial true plasma, β_A is equal to 76.8 mEq./L-pH and β_{gA} is equal to 52.6 mEq/L-pH. *In vivo,* the respiratory system controlling alveolar ventilation (and thus the pCO_2) represents β_g. Essentially the respiratory system acts by liberating H_2CO_3 when an acid is added and by accumulating H_2CO_3 when an alkali is added. Thus, if alveolar ventilation is not changed, β_A represents the total blood buffer value and occurs when the respiratory system does not respond to changes in pH.

If the alveolar ventilation is controlled so that there is no change in pH, then the values of $\frac{d\ \text{Total } CO_2}{d\ pH}$, $\frac{d\ H_2CO_3}{d\ pH}$, β_{gR}, β_g, and β become infinite. This would occur if the respiratory system is sensitive to changes only in pH and not in pCO_2. The value of β_{gR} represents the ventilation adjustment *in vivo* and actually depends upon the sensitivity of the respiratory system to pH and pCO_2. In order to determine β_{gR}, it is necessary to know the *in vivo* relationship between H_2CO_3 and pH. This relationship (produced by a nonrespiratory disturbance) has been determined by Gray[14] and, expressed in units appropriate to this development, is:

$$pH = 9 - \log \frac{5.48 + 82\ (H_2CO_3) - 39.5\ (H_2CO_3)^2}{H_2CO_3} \tag{18}$$

By differentiation, $\frac{d\ H_2CO_3}{d\ pH}$ can be obtained and then the value of β_{gR} can be found by substitution into equation (17):

$$\beta_{gR} = \frac{2.3\ (HCO_3^-)\ [5.48 + 82\ (H_2CO_3) - 39.5\ (H_2CO_3)^2]}{5.48 + 39.5\ (H_2CO_3)^2} \tag{19}$$

The value of β_{gR} is equal to 41.6 mEq/L-pH. Table 1 summarizes the

various components of the buffer value in blood and illustrates that most of the buffering is done by the respiratory system.

Addition or removal of H_2CO_3. The H_2CO_3 can be increased by a decreased alveolar ventilation or by an increase in carbon dioxide production by the cells or a combination of both. (Or of course, by an increase of

TABLE 1. COMPONENTS OF THE BUFFER VALUE OF HUMAN BLOOD

System	β in mEq/L-pH	% of total	
		No vent. adjust.	With vent. adjust.
Total chemical buffers of plasma in whole blood (β_{tc})	24.2	31	20
Plasma bicarbonate (β_b)	2.6	3	2
Excluding bicarbonate (β_c)	21.6	28	18
Red blood cell phase	16.2	21	14
Plasma phase	5.4	7	4
Plasma protein	5.0	6	4
Plasma phosphate (β_p)	0.4	1	0
Respiratory system (β_g)	94.2	...	80
No ventilation adjustment (β_{gA})	52.6	69	45
Change in ventilation (β_{gR})	41.6	...	35
Blood buffer value with no ventilation adjustment (β_A)	76.8	100	65
Total blood buffer value with ventilation adjustment (β)	118.4	...	100

the environmental carbon dioxide.) Equation (1) must still be valid even after addition of H_2CO_3. The net addition of H_2CO_3 (represented by d G) will result in an actual change in the H_2CO_3 and HCO_3^-, so that, neglecting carbamino formation (which is very small at a constant partial pressure of oxygen[2]), the following must hold:

$$d\,G = d\,H_2CO_3 + d\,HCO_3^- \tag{20}$$

Differentiation of equation (20) with respect to pH, results in:

$$\beta_{ha} = -\frac{d\,G}{d\,pH} = -\frac{d\,H_2CO_3}{d\,pH} - \frac{d\,HCO_3^-}{d\,pH} \tag{21}$$

where β_{ha} is the buffer value when the acid involved is H_2CO_3. Equation (4) can be rewritten as:

$$HCO_3^- = -\,a\,pH + b - X \tag{22}$$

where X is the concentration of a nonvolatile acid added. The value of $\frac{d\,HCO_3^-}{d\,pH}$ can be obtained by differentiating equation (22) with respect to pH:

$$\frac{d\,HCO_3^-}{d\,pH} = -\,a - \frac{d\,X}{d\,pH} \tag{23}$$

Differentiation of the Henderson-Hasselbalch equation (6) with respect to pH gives:

$$\frac{d\,H_2CO_3}{d\,pH} = \frac{H_2CO_3}{HCO_3^-}\,\frac{d\,HCO_3^-}{d\,pH} - 2.3\,H_2CO_3 \tag{24}$$

The resulting equations obtained by substitution of equation (23) into equations (21) and (24) can be combined to yield:

$$\beta_{ha} = \left(1 + \frac{H_2CO_3}{HCO_3^-}\right)\left(a + \frac{d\,X}{d\,pH}\right) + 2.3\,(H_2CO_3) \tag{25}$$

Equation (25) can be simplified to:

$$\beta_{ha} = \left(a + \frac{d\,X}{d\,pH}\right)\frac{Total\ CO_2}{HCO_3^-} + 2.3\,(H_2CO_3) \tag{26}$$

If a pH increase due to a decrease in H_2CO_3 is greater than a pH decrease due to an addition of a nonvolatile acid, then there will be a net increase in pH; and thus the value of $\frac{d\,X}{d\,pH}$ will be positive and add to the buffer value. The value of $\frac{d\,X}{d\,pH}$ will be positive when there is either a net removal of a nonvolatile acid in the presence of a decreased pH or a net addition of a nonvolatile acid in the presence of an increased pH.

For the situations in which no nonvolatile acids (or alkalies) are added, then $\frac{d\,X}{d\,pH} = 0$ and equation (26) becomes:

$$\beta_h = a\,\frac{Total\ CO_2}{HCO_3^-} + 2.3\,(H_2CO_3) \tag{27}$$

where β_h is the buffer value when the acid involved is H_2CO_3 and no nonvolatile acids (or alkalies) are added. For average arterial true plasma,[3] a = 21.6 mM/pH, HCO_3^- = 24.0 mM, H_2CO_3 = 1.2 mM, and Total CO_2 = 25.2 mM. Thus, β_h = 25.4 mEq/L-pH. For a bicarbonate solution, containing the same CO_2 concentrations, the value of a is zero, and thus the value of β_h is then equal to only 2.8 mEq/L-pH. Again the importance

of a, which represents the buffer value of chemical buffers excluding bicarbonate, is demonstrated in buffering.

If there is also a net removal (or gain) of nonvolatile acid caused by either the tissue cells or kidney or both, then the value of $\frac{d\,X}{d\,pH}$ does not equal zero, and can approach infinity (when there is no change in pH). Although, the kidney cannot remove acid rapidly enough to overcome an effect due to an acute respiratory disturbance (producing a H_2CO_3 change), it is to be pointed out that given a sufficient period of time, the buffer value due to the kidney can approach infinity (as evidenced by a normal pH in spite of a sustained respiratory disturbance). Such a situation occurs in the response to high altitude when the alveolar ventilation is increased, but the kidneys have eliminated sufficient bicarbonate until essentially the pH is returned to about normal.[8] Also in chronic pulmonary insufficiency, the data indicate that the pH is within a normal range in spite of high partial pressure of carbon dioxide.[18]

Factors concerned in the in vivo regulation of the pH of blood plasma. The relationship between the alveolar ventilation and the pCO_2 is given by[16]:

$$\dot{V}_A = \frac{863\,R\,\dot{V}_{O_2S}}{P_{A_{CO_2}}} \tag{28}$$

where $\dot{V}_A$ is the (expired) alveolar ventilation in ml/min, $\dot{V}_{O_2S}$ is the oxygen uptake in ml/min (corrected to standard condition, i.e., temperature = 0° C. and pressure = 1 atm), $P_{A_{CO_2}}$ is the alveolar partial pressure of CO_2, and R is the ratio of the carbon dioxide output in ml/min to the oxygen uptake in the same units. This equation assumes that the pCO_2 of the environment is equal to zero. If the carbon dioxide output is constant, then the product (R) ($\dot{V}_{O_2S}$) is constant, and thus the alveolar ventilation is inversely proportional to the $P_{A_{CO_2}}$. Factors which tend to prevent equilibrium between arterial blood and the gas alveolar phase[16] are (i) diffusion, (ii) venous arterial shunts, and (iii) unequal ventilation-blood flow ratios in the various alveoli. These factors will tend to increase the concentration of the physically dissolved carbon dioxide in the arterial blood assuming equilibrium with the alveolar tension. Actual measurements do not demonstrate any carbon dioxide change,[19] but the reason for this is that the method for detecting such a change is not sensitive enough. (The actual concentration of the physically dissolved gas is many times recorded as the partial pressure of the gas in a gas phase which would be in equilibrium with the liquid phase.) Thus, within experimental error, the $P_{A_{CO_2}}$ can be

assumed to be in equilibrium with the arterial blood, and thus the H_2CO_3 (in the arterial plasma) will be proportional to the $P_{A_{CO_2}}$.

During the unsteady state in which the carbon dioxide output is not constant, then the simple reciprocal relationship between the $P_{A_{CO_2}}$ and $\dot{V}_A$ will not be valid. The actual concentration of H_2CO_3 in the plasma will depend upon the rate of release of the H_2CO_3 from the large body carbon dioxide reservoir[7] and the rate of removal of the H_2CO_3, mainly by the respiratory system. If the respiration is stopped for a short time (breath holding), then the arterial concentration of H_2CO_3 will increase exponentially to the venous concentration of H_2CO_3.[5] Under physiological conditions, steady states of carbon dioxide occur relatively rapidly due to the rapidity of the respiratory adjustments and the easily available tissue carbon dioxide stores.[7]

Shifts of hydrogen ions (either dissociated or nondissociated) can occur across cell membranes and account for a considerable amount of buffering. In order to preserve electrical neutrality, a shift of hydrogen ions into a cell must also be accompanied by a shift of anions out of the cell or a shift of cations into the cell or some combination of these shifts. The hydrogen ion once inside the cell could be removed by buffers, such as protein, which would accept hydrogen ions rapidly. The rate of removal of hydrogen ions by the cells would therefore be limited by the rate of ion transport across the cell membrane. For the red blood cell, the ion shift equilibrates in a couple of seconds and is due to chloride ions[4]; whereas for muscle cell, the ion shift is due mainly to potassium ions.[10] There is also a sodium ion shift from bone and tendon.[15] The following equation can be used to obtain an estimate of the total chemical buffer value of the body:

$$\beta_B = \frac{(\beta_{tc})(V_b) + (\beta_t)(V_t)}{V_b + V_t} = \frac{\beta_{tc} + (\beta_t)(S)}{1 + S} = \frac{\beta_{tcb}}{1 + S} \qquad (29)$$

where β_B is the total chemical buffer value of the body per unit volume of total body water, β_t is the chemical buffer value of tissue (excluding bicarbonate), V_b is the blood water volume, V_t is the tissue water volume (including interstitial water), S is the ratio of V_t to V_b, and β_{tcb} is the total chemical buffer value of the body per unit volume of blood water. In obtaining the percentage contributions of the various buffer components, it is important that each buffer value component is calculated using the same volume. Although the total buffer value will depend upon the volume used, the percentage contribution of the buffer components will not depend upon the volume. It has been estimated that the tissue phase contributes about five times as much as the blood phase in buffering.[17, 22] Thus, the ratio of $(\beta_t)(S)$

to β_{tc} is equal to 5. Since about 8 per cent of the body is composed of blood[23] (8 liters blood per 100 kilogram body weight) and since 80 per cent of the blood is water, it follows that 6.4 per cent of the body is blood water (V_b). About 70 per cent of the body is composed of water ($V_b + V_t$),[11] and so V_t is about 63.4 per cent. The value of S is therefore about 10, and so the ratio of β_t to β_{tc} must be equal to about 0.5. The measured chemical buffer values (excluding bicarbonate) of frog muscle and blood are respectively 6.1 mEq/kg-pH and 17.2 mEq/L-pH,[8] which would indicate that the ratio of β_t to β_{tc} would be about 0.3. However, as pointed out earlier, other tissues, such as bone, also participate in buffering, and therefore the value of 0.5 seems reasonable. Since β_{tc} is 24.2 mEq/L-pH, the estimated value of β_t is 12.1 mEq/L-pH and β_B is therefore 13.2 mEq/L-pH. The value of β_B is the weighted average of the buffer values of blood and tissue, and shows that the chemical buffering efficiency is much less for the whole body than for the blood. However, the buffer value per unit volume of blood water (β_{tcb}) is greater than either component (β_{tc} or β_t) and equals 6 β_{tc} or 145.2 mEq/L-pH. Thus, these calculations illustrate that although the blood is better buffered than the tissues, the tissues contribute more to the buffering of the body because of their relatively large volume.

The total buffer value of the body must also take into consideration the role played by the respiratory system and kidneys:

$$\beta_T = \frac{\beta_{tcb} + \beta_g + \beta_k}{1 + S} = \beta_B + \frac{\beta_g}{1 + S} + \frac{\beta_k}{1 + S} \qquad (30)$$

where β_k is the buffer value of the kidneys per unit volume of blood water and β_T is the total buffer value of the body per unit volume of body water. In calculating the total buffer value of the body per unit volume of body water, it is implied that there is an equal concentration of acids (and alkalies) across the cell membranes of the tissues. If the total buffer value of the body per unit extracellular volume is used, then it is implied that the acids (and alkalies) cannot diffuse into the cells. Actually, neither implication is correct, and the situation will depend upon the actual acid (and alkali) studied. The relation between the total buffer values using the different volumes is:

$$\beta_E = \frac{(V_b + V_t)}{V_E} \beta_T \qquad (31)$$

where β_E is the total buffer value of the body per unit extracellular volume and V_E is the extracellular volume (20 per cent of the body[11]). As pointed out before, the buffer value of the kidneys can approach infinity under certain conditions. If the role of the kidneys is neglected (as in nephrectomy)

and if nonvolatile acid is administered, then Table 2 illustrates the components of the total buffer value of the body per unit volume of body water at equilibrium and is compatible with experimental data.[19, 20] The value of β_E is calculated to be 76.2 mEq/L-pH using the value of 21.8 mEq/L-pH for β_T (Table 2). It is evident that the chemical buffers of the blood only contribute a small percentage to the buffer value of the body, whereas the chemical buffers of the tissues contribute one-half of the total. Again, it should be emphasized, that these calculations assume equilibrium, but that the blood cells react rapidly whereas the tissue cells react much more slowly.

TABLE 2. COMPONENTS OF THE TOTAL BUFFER VALUE OF THE BODY PER LITER BODY WATER (EXCLUDING KIDNEY COMPONENT)

System	*β in mEq/L-pH*	*% of total*
Total chemical buffers of body (β_B)	13.2	61
Blood $\left(\frac{\beta_{tc}}{1+S}\right)$	2.2	10
Tissue $\left(\frac{(\beta_t)(S)}{1+S}\right)$	11.0	51
Respiratory system $\left(\frac{\beta_g}{1+S}\right)$	8.6	39
Total buffer value per liter body water (excluding kidney component) (β_T)	21.8	100

Neglecting the role of the kidneys and neglecting chemical absorption of carbon dioxide (carbamino formation), then an estimation of the total buffer value of the body per unit volume of body water (β_{T_h}) to the retention of H_2CO_3 can be obtained using equation (26):

$$\beta_{T_h} = \frac{\beta_c + (\beta_t)(S)}{1+S} \frac{\text{Total } CO_2}{HCO_3^-} + \frac{2.3\ (H_2CO_3)}{1+S} \qquad (32)$$

The value of β_{T_h} is calculated to be 13.9 mEq/L-pH, the component due to the tissues is 11.6 mEq/L-pH and the component due to the blood is only 2.3 mEq/L-pH. Thus, again the tissues have a buffer value five times greater than that of blood. *In vivo* experiments have demonstrated the importance of the tissues in buffering.[12] Actually β_{T_h} represents an average of the blood and tissue buffer values. Due to the unequal distribution of ions across the membranes of cells,[1] anomalous ion shifts can occur. Thus if the red cell phase is increased, then it is possible that the chloride shift due to addition of H_2CO_3 could increase the plasma HCO_3^- to such an extent

that in spite of an increase of the bicarbonate in the tissue phase (due to addition of H_2CO_3), there will be a net tendency for the bicarbonate to enter the tissues along with some cation. This can explain the observation that when muscle cells are immersed in blood and equilibrated with carbon dioxide, there is a potassium shift from the plasma into the muscle cells.[9]

In summary, the buffering of blood *in vivo* is dependent upon the relatively rapid mechanisms (the first line of defense), involving the respiratory system and the red blood cells, and the relatively slow mechanisms (the second line of defense), involving the kidneys and the tissue cells.

CONCLUSIONS

At a constant pCO_2, the total blood buffer value is 76.8 mEq/L for a change of one pH unit. Of this total, 3 per cent is due to the plasma bicarbonate, 28 per cent is due to other chemical buffers in the blood, and 69 per cent is due to the presence of a gas phase.

When there is a change in the alveolar ventilation due to the response of the respiratory system to pH and pCO_2, then there is a substantial increase in the blood buffer value.

The tissues and kidneys have the capacity to contribute substantially to the buffer value of blood, but they react more slowly than the respiratory system and the chemical buffers in the blood.

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HOWARD LEVITIN*
ANDRZEJ MANITIUS**
FRANKLIN H. EPSTEIN†

Department of Internal Medicine, Yale University School of Medicine

URINARY DILUTION IN POTASSIUM DEFICIENCY‡

One of the earliest manifestations of potassium depletion is a reduction in the ability of the kidneys to elaborate a concentrated urine.[8] This defect has been shown to result at least in part from a decrease in the concentration of sodium in the interstitial fluids of the renal medulla and papilla.[9] The disturbance in renal concentrating ability may exist without impairment of glomerular filtration rate; renal extraction of para-aminohippurate[10] and the ability of the kidneys to excrete a highly acid urine, however, are frequently deficient.[3]

The present experiments have explored the ability of potassium-deficient animals to excrete a *dilute* urine in response to a water load. In contrast to certain other diseases of the kidney accompanied by hyposthenuria, the kidneys of potassium-depleted animals appear to dilute the urine normally. Studies of urinary dilution and concentration in two patients with severe potassium deficiency are also reported.

METHODS

Twenty-one white male Sprague-Dawley rats weighing 170-220 grams were divided into experimental and control groups. Ten rats comprised the experimental group and were depleted of potassium by diet and desoxycorticosterone (DCA). Eleven rats served as controls and were fed a normal diet throughout the experiment. Both groups were studied concomitantly.

Following an initial seven days of normal diet, both groups were subjected to a water-loading test. Water equal to 5 per cent of body weight was given in a single dose by gastric intubation and the animal placed in a metabolic cage. During the next two to three hours, urine was collected in three consecutive aliquots of at least 2.5 ml. and the osmolality of each sample determined with the Fiske osmometer. Twelve hours later, drinking water was withdrawn. After 12 hours of water deprivation, 50 milliunits of vasopressin in oil were injected subcutaneously. The rats usually voided spontaneously

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* Advanced Research Fellow of the American Heart Association; Instructor in Medicine.
** Special Fellow of the Rockefeller Foundation.
† Established Investigator of the American Heart Association; Associate Professor of Medicine.
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while they were being injected. They were then placed in metabolic cages over funnels filled with mineral oil to minimize evaporation, and urine was collected during the next 12 hours for the determination of maximum urinary osmolality.

Thereafter the first group of rats was depleted of potassium by a diet containing normal amounts of sodium but low in potassium[9] and by daily subcutaneous injections of 0.2 mg. DCA for seven days. DCA was then discontinued and the low-potassium diet

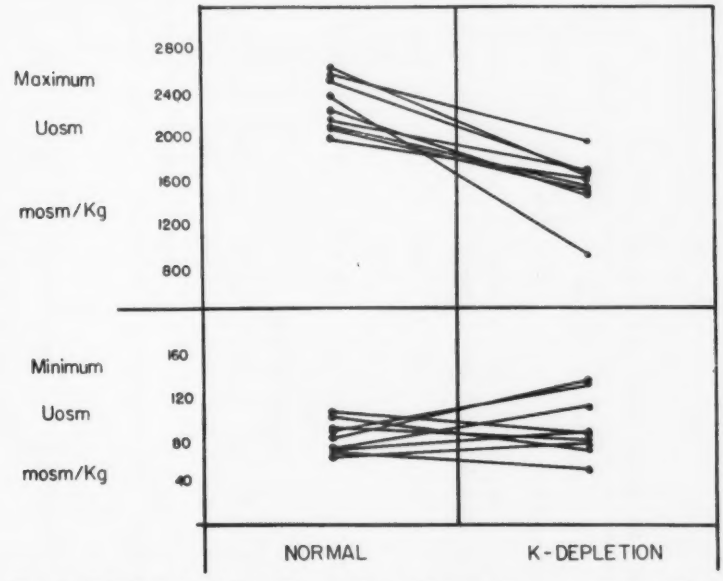

FIG. 1. Potassium deficiency did not alter *minimum* urinary osmolality, although *maximum* urinary concentration was greatly diminished.

maintained for the remainder of the experiment. Two days after stopping DCA both groups of animals were again gavaged with water to assess their diluting ability, and this was followed 36 hours later by a concentration test. At the conclusion of the experiment the rats were anesthetized with pentobarbital, exsanguinated via the abdominal aorta, and specimens of thigh muscle were obtained.

The chemical techniques employed for the analysis of tissue, urine and plasma were those previously detailed.[9]

RESULTS

Potassium depletion resulted in the expected decrease in the content of potassium of serum and muscle and a slight increase in muscle sodium (Table 1). Maximum concentrating ability was greatly diminished, U_{max} falling from an average of 2258 mosm/Kg. to 1570 mosm/Kg.

TABLE 1. THE EFFECT OF POTASSIUM DEPLETION ON URINARY CONCENTRATION AND DILUTION IN RATS

	Experimental (10 rats)										
	Initial				*Final*						
	Wt. gm.	*Max. U_{osm} mosm/Kg.*	*Min. U_{osm} mosm/Kg.*	*U_{Na} meq/L*	*Wt. gm.*	*Max. U_{osm} mosm/Kg.*	*Min. U_{osm} mosm/Kg.*	*U_{Na} meq/L*	*Serum K meq/L*	*Muscle K meq/100 gm. FFDS**	*Muscle Na meq/100 gm. FFDS**
Mean	188	2258	85	5.5	211	1570	94	5.8	2.7	39.7	13.9
s.d.	14	266	31	3.0	23	266	27	2.4	0.6	2.1	1.2
p (Initial vs. Final)						< 0.01	> 0.4	> 0.7			
	Control (11 rats)										
Mean	191		102	7.8	223	2048	104	7.1	4.0	44.9	8.9
s.d.	14		27	4.5	14	249	52	5.4	0.4	1.7	1.7
p (Exp. vs. Control)			> 0.2	> 0.2		< 0.01	> 0.6	> 0.5	< 0.01	< 0.01	< 0.01

* Fat-free dry solids.

Despite the evident loss of the ability of the kidneys to concentrate urine, diluting ability remained unimpaired (Fig. 1). Minimum urinary osmolality was essentially unchanged by potassium deficiency (Table 1), and the time required by depleted rats to excrete a water load was not significantly different from that of normal controls (Fig. 2). Although a tendency to waste sodium in the urine has been demonstrated in hydropenic potassium-

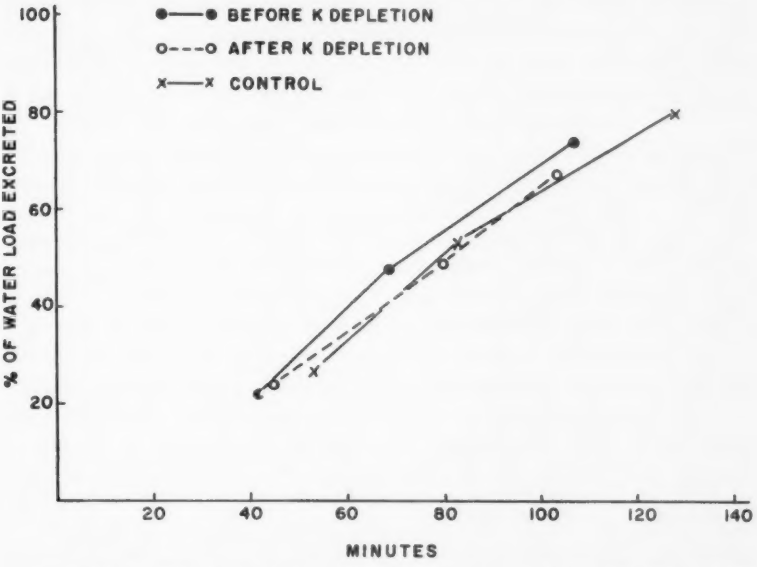

FIG. 2. Time required by rats to excrete a water load equal to 5 per cent of body weight. The ability to excrete water was unaffected by potassium depletion.

depleted rats on a low-sodium diet,[9] water-loaded animals in the present experiments, on a normal-sodium diet, excreted the same amount of sodium in the urine before and after potassium depletion.

Water-loading experiments were carried out on two patients severely depleted of potassium, as judged from subsequent balance studies carried out during repletion (Table 2). M.A., with nephrocalcinosis and renal potassium wasting, could concentrate the urine only to 325 mosm/Kg. after the intramuscular administration of vasopressin in oil. Creatinine clearance, blood nonprotein nitrogen and phenolsulfonphthalein excretion were all within the normal range. Although potassium-deficient and unable to concentrate the urine, she excreted 70 per cent of a liter of ingested water within

three hours and attained a minimum urinary osmolality of 45 mosm/Kg., a normal response. On the other hand, H.B., a middle-aged lady depleted of potassium by laxative addiction, initially exhibited much more severe impairment of renal function, with an NPN of 52 mg./100 ml. and a

TABLE 2

Patient	*M.A. ♀ 46 yrs. 45 Kg.*		*H.B. ♀ 53 yrs. 52 Kg.*
Diagnosis	*Renal tubular acidosis, nephrocalcinosis, renal K-wasting*		*Laxative addiction*
Water load	Low-K	1,000 ml. p.o.	1,000 ml. i.v.
	After repletion	1,000 ml. p.o.	1,000 ml. p.o.
Excreted in 3 hrs. ml.	Low-K	700	455
	After repletion		455
Min. U_{osm} mosm/L	Low-K	45	143
	After repletion		135
Max. U_{osm} mosm/L	Low-K	280	218
	After repletion	545	718
NPN mg/100 ml.	Low-K	32	52
	After repletion		42
C_{cr} ml/min.	Low-K	75	
	After repletion	88	
PSP % in 2 hrs.	Low-K	82	31
	After repletion		77
Serum K meq/L	Low-K	2.1	2.5
	After repletion	4.5	4.6
Estimated* deficit of K meq		540	700

* From balance studies during repletion.

phenolsulfonphthalein (PSP) excretion of only 31 per cent in two hours. Maximum urinary concentration after intramuscular injection of five units of vasopressin in oil was only 218 mosm/Kg. One liter of 5 per cent glucose in water given intravenously was excreted abnormally slowly, only 45 per cent appearing in the urine after three hours. The lowest urinary osmolality achieved was 143 mosm/Kg. Diluting ability did not improve appreciably even after potassium balance was restored. Impaired urinary dilution in this

potassium-depleted patient was probably associated with restriction of glomerular filtration rate and renal blood flow, perhaps as a result of more intense or prolonged generalized renal injury by potassium deficiency.

DISCUSSION

These experiments demonstrate that potassium deficiency in rats, severe enough to produce marked impairment of renal concentrating ability, need not interfere with the capacity of the kidneys to dilute the urine in response to a water load. The results are consistent with the report of Brokaw that the time required for rats to excrete half of an oral water load consisting of 5 per cent of body weight was not prolonged by potassium depletion, although elimination of much larger doses of water was slowed.[3]

Urine is presumably diluted by active reabsorption of sodium in the thin loop of Henle and the distal convoluted tubule.[4, 12] The present experiments suggest that this process is not greatly disturbed by potassium depletion. This conclusion is reinforced by the finding of Gottschalk that fluid obtained by micropuncture of the distal convoluted tubule in potassium-deficient rats is as dilute as that obtained from the same site in normal animals.[5] These data are of interest in the light of the increased urinary sodium and decreased content of sodium of the renal medulla and papilla of hydropenic potassium-deficient animals.[9] Although they do not exclude the possibility that active reabsorption of sodium by medullary loops of Henle is diminished by potassium deficiency, they suggest that other mechanisms may be responsible for the lowered gradient of sodium concentration between cortex and papilla which characterizes this condition.

The ability to excrete a dilute urine depends not only upon the reabsorptive capacities for sodium of the ascending loop of Henle and the distal convoluted tubule, but also upon the quantity and composition of fluid delivered to these sites. The excretion of free water may be expected, therefore, to vary with the rate of glomerular filtration.[7] Thus, although moderate polyuria may characterize individuals with renal insufficiency and azotemia who have lost the ability to concentrate their urine, delayed excretion of water and decreased ability to dilute the urine is also generally the rule in such patients. Whether a particular patient with a deficit of potassium is able to excrete a normally dilute urine probably depends upon the degree to which glomerular filtration has been impaired as well as upon the extent of associated lesions of the renal parenchyma. It is interesting that patient M.A., with comparatively little restriction of endogenous creatinine clearance and a normal phenolsulfonphthalein excretion, was able to dilute her urine normally, while H.B., with considerably more severe impairment of renal

function, and a greater over-all deficit of body potassium, exhibited some restriction of water diuresis which persisted even after the body stores of potassium were repleted. Rubini has reported that normal human subjects in whom mild degrees of potassium depletion have been induced under controlled conditions retain the ability to dilute the urine at a time when concentrating power is distinctly diminished.[11]

Retention of the ability to excrete a dilute urine is probably responsible for the prominence of severe polyuria in some patients with potassium deficiency, especially those in whom disturbances of thirst or posterior pituitary secretion exist which lead to excessive drinking.[8,9]

SUMMARY

1. Potassium depletion severe enough to lower the content of potassium in serum and muscle and to impair renal concentrating ability was induced in rats.

2. The capacity of the kidneys to excrete a dilute urine in response to an oral water load was unaltered by potassium deficiency.

3. Renal diluting ability was also normal in one patient with severe potassium deficiency but was found to be restricted in another.

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Apparatus for Continuous Monitoring of the Fetal Heart Rate.*

By Edward H. Hon, Department of Obstetrics and Gynecology, Yale University School of Medicine.

During the past four years continuous monitoring of fetal heart rate during labor and delivery has permitted the accumulation of accurate, permanent records which may be of value for the assessment of fetal heart rate as an index of fetal environment.

The QRS complex of the fetal ECG has been used as a trigger source for a cardiotachometer and oscillograph system.[1-3] The major difficulty has been the elimination of the maternal ECG from the combined maternal and fetal ECG tracing. Various separation methods have been used with some success; the most promising is the attachment of a small electrode to the fetal scalp or buttocks. This method has been used for over a year and

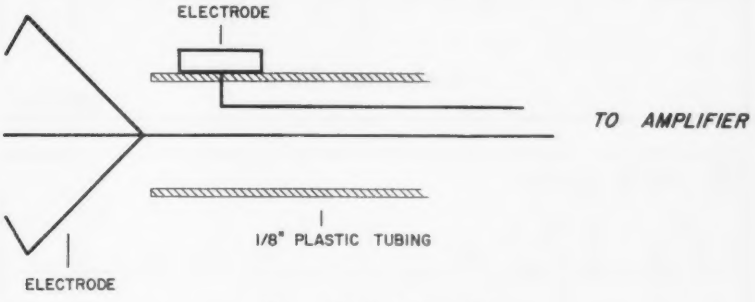

Fig. 1. Vaginal electrode.

has provided continuous, accurate records throughout labor and delivery. The original research electrodes had minor mechanical and electrical disadvantages and occasionally were difficult to apply.[4]

With the co-operation of an electronic instrument manufacturer, an electronically efficient and mechanically stable vaginal electrode has been developed (Fig. 1). The central stainless steel wire fingers penetrate superficially the fetal epidermis and the other electrode of the bipolar system makes electrical contact with the adjacent areas of the fetus via the vaginal and cervical secretions. To minimize electrical "noise" the entire electrode assembly is shielded and the patient's leg is grounded.

Figure 2A is an example of the type of fetal ECG tracing obtained with the vaginal electrode attached to the scalp of a fetus during active labor. Fetal P and T waves and QRS complexes (average 50-100 microvolts) are

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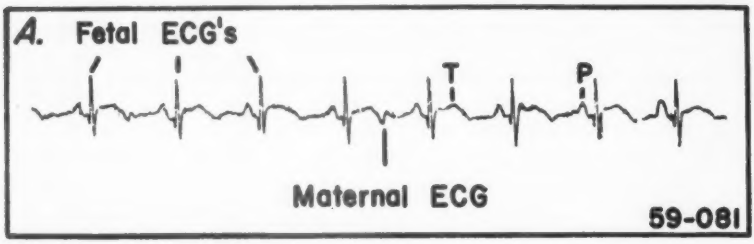
seen clearly. The maternal ECG is of low amplitude and does not interfere in any way with automatic counting of the fetal heart rate.

A combined fetal and maternal ECG tracing recorded from the abdominal wall of a pregnant patient is shown in Figure 2B as a basis of comparison of this type of recording with that obtained with the vaginal electrode. With the abdominal electrode the maternal ECG is of greater amplitude than the fetal ECG (10-25 microvolts), thus necessitating some type of signal conditioning to remove the maternal ECG before the fetal heart rate can be counted



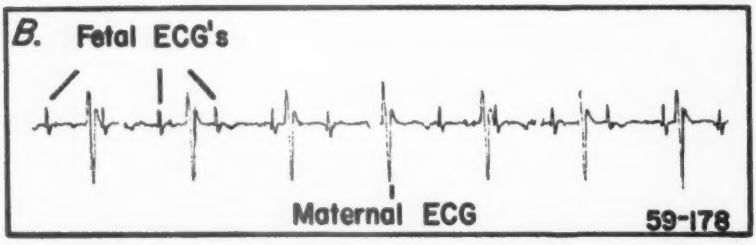


FIG. 2A. Fetal ECG tracing recorded from an electrode attached to the fetal scalp. Note fetal P and T waves and sporadic low amplitude maternal ECG.

FIG. 2B. Tracing of maternal and fetal ECG's as recorded from electrodes attached to the abdominal wall of a patient. Maternal ECG is of greater amplitude than the fetal ECG.

automatically. The fetal P and T waves are of low amplitude and are unrecognizable in the baseline electrical "noise."

The vaginal electrode permits continuous monitoring of fetal heart rate with relatively simple apparatus. In addition, the presence of recognizable fetal P and T waves are of value in studying fetal ECG configuration changes and cardiac arrhythmias.

The electrode is coupled to a high gain differential amplifier which has in-built filters. The filtered output is counted by a cardiotachometer which provides a numerical display and an output voltage which is plotted by an oscillograph. Provision is also made for continuous recording and oscilloscope monitoring of the fetal ECG.

The commercial* versions of the electrode and fetal heart rate monitoring system are more efficient and reliable than the research apparatus constructed in our laboratories. The electrodes are inexpensive and disposable.

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* Epsco Medical, Cambridge, Mass.

BOOK REVIEWS

HUMAN BIOCHEMICAL GENETICS. By H. Harris. New York, Cambridge University Press, 1959. 310 pp., 50 tables, 67 figs. $7.00.

There are few areas of knowledge whose continued expansion and increasing applicability can be as confidently predicted as is the case for human biochemical genetics. A growing awareness of the importance of this field has resulted in a rapid increase in the number and quality of studies contributed from several disciplines. Under these circumstances Dr. Harris' book renders the timely service of taking stock of our present situation. It is a worthy successor to the pioneer publications of A. E. Garrod and to Dr. Harris' own summary in 1953.

The first two chapters provide a framework in the form of a compact and lucid presentation of the principles and the physical basis of Mendelian heredity, ranging from cryptogenetics to the dynamic aspects of population genetics. The bulk of the text deals with heritable biochemical defects of amino acid metabolism, carbohydrate metabolism, the hemoglobins, the blood group substances and the plasma proteins. Genetic abberations in porphyrin metabolism, and a number of miscellaneous conditions, are treated in a separate chapter. A final chapter on the problem of gene action is most provocative and stimulating.

The instances which Dr. Harris has chosen to detail, although greater in number than in his *Introduction to Human Biochemical Genetics* (1953), have been selected with the same discrimination. As a result, the book is a showcase of important conditions that have been extensively studied and well documented. In the aggregate they convey a picture of the status of the field today. Each instance has been treated in breadth. The clinical, chemical, and genetic aspects are expertly introduced. In addition to the usual discussions of modes of transmission, the text contains pertinent information concerning geographic distribution, incidence, and mutation rate. The references which supplement the text are both extensive and excellent.

Dr. Harris has produced an authoritative and clearly written summary which will command the attention and the gratitude of the clinician, the biochemist, and the geneticist.

ALFRED J. COULOMBRE

STRESS AND CELLULAR FUNCTION. By H. Laborit. Philadelphia, J. B. Lippincott Co., 1959. 255 pp. $7.50.

This book entitled *Stress and Cellular Function* has the laudable purpose of correlating disease with specific defects in cellular metabolism. Unfortunately, only one of the sixteen chapters concerns itself with cell physiology and this one is largely restricted to a discussion of the diffusion of ions across the membranes of various cell organelles. The next few chapters extend the ion discussion to the pulmonary and renal organ systems and correlate the movements of hydrogen ions with altered metabolic states.

The major portion of the book is devoted to a defense of hypothermia and artificial hibernation as a desirable form of therapy for a variety of disease states. Many physiological studies are cited to support this approach and many hypotheses regarding the effect of hypothermia on organs

and cells of the body are proposed. In this reviewer's opinion, it is doubtful if sufficient knowledge exists at the present time to justify the suggested correlations.

DONALD W. KING

CELL, ORGANISM AND MILIEU. Dorothea Rudnick, Ed. New York, Ronald Press, 1959. 326 pp. $8.00.

This Seventeenth Growth Symposium volume is comprised of papers presented in June of 1958. As stated in the foreword, the theme of the conference was that of "Differentiation and growth in response to a changing chemical environment." As Dr. Rudnick points out, and as is all too obvious, this permits an extremely wide range of papers. Subjects and authors are: (1) Wilde—Differentiation in response to chemical environment; (2) Hoffmann-Berling—Cell structures in cell movements; (3) Nason—Tocopheral in muscle; (4)—Csapo—Myometrial regulation; (5) Moog—Phosphatase adaption; (6) Schneiderman—Insect growth hormones; (7) Torrey—Modification of root development; (8) Luckwill—fruit growth in relation to chemical stimuli; (9) Loomis—$C0_2$ and feedback mechanisms; and (10) Lutwak-Mann—Physiology of mammalian blastocyst. Treatments vary greatly, from very detailed accounts of limited subjects, as the Tocopheral paper, to more generalized talks, such as Wilde's, to excellent theoretical discussions, such as the Loomis $C0_2$ story. Because of the wide range dealt with it is difficult to recommend this volume to any one type of biologist, or even to a developmental biologist in general, for with depth of treatment in very specific subjects comes less general applicability and interest. Nevertheless, this volume, like the preceding Growth Symposia, does present a sampling of the broad range of activity that interests modern developmental biologists.

N. K. W.

ELECTROLYTES IN BIOLOGICAL SYSTEMS. Abraham M. Shanes, Ed. Washington, American Physiological Society, 1955. Distributed by the Ronald Press Co., 15 East 26 St., New York 10, N. Y. 243 pp. $4.00.

The active transport of inorganic ions across cell membranes is such a fundamental aspect of life itself that its study is a common meeting ground for plant physiologists, general physiologists, renal physiologists, and biologists interested in nerve cells, muscle cells, red cells, intestinal mucosa, or, indeed, any kind of living cell.

This volume contains the proceedings of a symposium held at Woods Hole in 1954 on the transport of electrolytes in biological systems varying from yeast cells and salt water algae to frog skin, nerve tissue, and human red blood cells. The nine chapters constitute a good summary and bibliography of the subject up to that date, somewhat in the style of the comprehensive papers usually published in *Physiological Reviews*. They make a worthwhile starting place for the student who needs to begin *somewhere* before working forward through a literature which may be unfamiliar. The chapter by Hogben on biological aspects of active chloride transport and

that by Huf on ion transport in frog skin seem especially complete and clarifying to this reviewer.

FRANKLIN H. EPSTEIN

PHYSIOLOGY OF INSECT DEVELOPMENT. Frank L. Campbell, Ed. Chicago, Univ. of Chicago Press, 1959. 167 pp. $4.00.

The *Physiology of Insect Development,* edited by Frank L. Campbell, is one of ten volumes covering the "Developmental Biology Conference Series of 1956," organized by Paul Weiss and held under the auspices of the National Academy of Sciences-National Research Council. The purpose of this series was a critical examination and evaluation of the contributions from various fields of research to the understanding of fundamental problems in developmental biology, with the hope that a new orientation and new ideas might arise.

On the basis of this book, it appears that the conference on the physiology of insect development fell short of the proposed ideal, following more the pattern of a symposium, but with unrestrained discussions prompted by the material that the various specialists presented. The topics reviewed included insect embryology, larval development, tissue culture, metamorphosis, diapause, histolysis, tumors, and regeneration. Although the participants engaged in the discussions with enthusiasm, few new concepts seemed to emerge. The second session on embryology came closest to attaining the desired goal of the conference.

It is unfortunate that the editor of this volume chose to present it entirely in dialogue form. Even with pruning, the resulting text is often rambling, disorganized, teleological, and lacking in accuracy. This book may serve some purpose as a record of events occurring at the conference or as a stimulus for additional research along certain lines. It cannot be recommended as either a reference or textbook.

W. W. DOANE

THE HISTORICAL DEVELOPMENT OF PHYSIOLOGICAL THOUGHT. 1st Ed. Chandler McC. Brooks and Paul F. Cranefield, Eds. New York, The Hafner Publishing Company, 1959. 401 pp. $6.00.

As a part of programs designed to commemorate its centenary year and for the dedication of a new Basic Science Building a series of seminars was held at the Down State Medical Center of the State University of New York during the academic year 1956-57 on "The Historical Development of Physiological Thought." The lectures that formed the essential part of the seminars and two essays, one contributed by Sir John Eccles and the other by Dr. Harold A. Levy, form the substance of this volume. As might be expected in such a series of lectures given by distinguished contributors to specific areas of biological knowledge, there is a wide variety in the approach to the subject and in the breadth or narrowness of the aspect treated. Some of the lecturers appear to have paid little attention to the general theme of historical development, but the best reading, and one suspects that the writing in this volume which will endure longest, traces

for the advantage of the reader how personality and timeliness of ideas have been major forces in the growth of our understanding of the "Wisdom of the Body." Especially to be recommended to students interested in tracing the background of modern concepts in neurophysiology are the essays by Professor Horace W. Magoun on "The development of ideas relating the mind with the brain," and Professor John F. Fulton "Historical backgrounds of neurophysiology." In the latter Dr. Fulton draws effectively on his associations with Sherrington and Pavlov to fix their respective roles in the growth of our understanding of behavior. Dr. Chandler Brooks, one of the editors of the volume, has contributed an excellent review of the stages in "The discovery of the function of chemical mediators in transmission of excitation and inhibition to effector tissues." For the student this review should have especial interest, for here he will see the finger-pointings to the truth a full thirty years before men's minds were prepared to accept an explanation that challenged naught but tradition.

This selection of specific essays from the group betrays the reviewer's special interests. They are all of high quality and each one certain to reward the reader.

DONALD H. BARRON

AMINE OXIDASE INHIBITORS. By D. Wayne Woolley (and others). Annals of the New York Academy of Sciences, vol. 80, art. 3, pp. 551-1045. New York, 1959. $5.00.

This volume includes the papers presented at a conference on inhibitors of monoamine oxidase (MAO) held at the New York Academy of Sciences on November 20-22, 1958. It presents reports ranging from structure-activity relationships of new series of MAO inhibitors to the clinical evaluation of these compounds; it should, therefore, have an appeal for a wide variety of workers in this area of research, from laboratory investigators to psychiatrists.

Most of the investigators at the conference agreed that the clinical properties of these compounds are interesting, promising, and worthy of further work. It would appear from their reports that these drugs have some potential value in the following clinical conditions.

1. *Psychiatric disorders.* This is probably the most important area of their application. Generally, chronic schizophrenics who are apathetic or depressed respond better than those with agitated depression or those classified as acute schizophrenics. In this latter group, as many as 75 per cent may be made worse by these agents. These are the findings of Bailey *et al.* who also report success in refractory patients by using a combination of an MAO inhibitor and a tranquilizer. A group from Boston (Alexander and Berkely) illustrated the important point that careful selection of patients for therapy with iproniazid can lead to a much higher percentage of success than poor selection. They reported a recovery rate of 88 per cent in a group classified as psychesthenic-anhedonic in contrast to only 26 per cent recovery among those exhibiting classical depressive reactions. A number of other papers on the efficacy of these drugs in psychiatric disorders have the same positive and optimistic tone, but it seems safe to

say that their ultimate place in psychiatry must await many more definitive studies. Voelkel of Berlin (Waldhaus Mental Hospital) made an interesting point when he generalized that the action of the hydrazides occurs in two phases: activity is affected first, followed by alteration of mood. Possibly the greatest single drawback to these drugs, apart from their toxicity, is their relatively slow onset of action. There is a good likelihood that more effective agents in this category will be introduced in the future.

2. *Angina pectoris and coronary disease.* Cesarman of the National Institute of Cardiology of Mexico, who first observed the beneficial action of iproniazid in angina pectoris, has indicated that this application was a clear case of serendipity; and today even hints as to the mechanism of this action are few and obscure. Nevertheless, all investigators at this conference were in agreement that good results may be expected from iproniazid and, presumably, from related compounds. There was also general agreement on the relief of pain and the mental changes produced by these compounds, such as euphoria, excitement, and loss of perception of the angina. On the other hand, there was disagreement about whether iproniazid can correct ECG-abnormalities and change the response to the Master exercise test. One important point which emerged from these reports is that one obtains no therapeutic results without attendant side-effects.

3. *Diseases of the gastro-intestinal tract.* In a number of conditions like colitis, hepatitis, anorexia nervosa, etc., in which patients suffer from lack of appetite and malnutrition, Weiss and co-workers reported improvement in appetite and sense of well-being after long-term therapy with iproniazid. Their data strongly suggest that the appetite-improvement is a direct result of a significant reduction in blood glucose.

4. *Rheumatoid arthritis.* An interesting paper by Scherbel and Harrison (Cleveland Clinic) demonstrated that iproniazid may have a place in the treatment of retarded psychomotor activity, emotional lability, and other CNS manifestations occurring in patients with rheumatoid arthritis and related diseases. It was made clear that the drug had no effect on the objective signs, nor did it alter the clinical laboratory data. But there was improvement in motility and in the subjective symptoms of these patients.

It becomes apparent from the clinical papers presented here and from those directly reporting toxicity that these drugs have a toxic potential of considerable proportions. The toxicity of iproniazid ranges from annoying side-effects like dizziness, urinary retention, and impotence to serious and sometimes fatal hepatocellular changes. In general, it is felt that serious toxicity can be avoided by keeping the dosage of iproniazid under 150 mg. daily.

While there is no question that these drugs influence the metabolism of amines within the organism, papers dealing with mechanism of action failed to present rigorous evidence to support the view that inhibition of MAO can account for all of the effects observed with these drugs. In the mind of the reviewer there is little reason to doubt that other mechanisms are also involved.

NICHOLAS J. GIARMAN

CURRENT TRENDS IN RESEARCH AND CLINICAL MANAGEMENT OF DIABETES. By Peter H. Forsham (and others). Annals of the New York Academy of Sciences, vol. 82, art. 2, pages 191-644. New York, 1959. $4.50.

The presence in the United States of one and a half million known diabetics, and an almost equal number of estimated unsuspected cases, is a sharp pointer to the importance of the disease as a public health problem. This series of papers is the result of a conference covering many aspects of the subject, held by the New York Academy of Sciences on April 10 and 11, 1959. The contributions are divided into four sections, dealing in turn with clinical facets of diabetes, advances in the physiology of insulin secretion, the biochemistry of insulin and sulfonylurea compounds, and the status of oral agents in therapy.

It may be said at once that there is ample food here for the most thoughtful appetite. This is exemplified in the opening paper by Steinberg, in which the evidence in favour of diabetes as a homozygous recessive condition is marshalled. It is also pointed out that, although diabetes appears earlier in the majority of diabetic children than in their diabetic parents, the frequency of prior onset could be due to chance, and there is no evidence of anticipation as a biological phenomenon. The value to genetic studies of being able to diagnose the prediabetic state is emphasised, and in the next paper Fajans and Conn present a possible answer to this problem in the cortisone-glucose tolerance test. Some good debating points are scored by Ellenberg in a survey of diabetic neuropathy. The occurrence of this symptom as a presenting feature reinforces his plea for its recognition as an integral manifestation of the disease, rather than a complication.

Four papers follow, dealing with islet-cell structure and function. Of particular interest is the finding by Volk and Lazarus that rabbits treated with hyperglycemic agents show degranulation but never destruction of the beta cells, and subsequently new beta cell formation occurs.

In a description of their technique of liver perfusion, Mortimore and Tietze observe that considerable inactivation of I^{131}-labelled insulin is brought about by this organ. Berson and Yalow review the whole question of insulin-binding antibodies in man, and discuss a method of assaying insulin, based on its reaction with a specific antiserum.

The results of Kipnis, who compares the glucose uptake by cut and intact rat diaphragm muscle, suggest that insulin acts principally on membrane transfer, whereas diabetogenic hormones diminish intracellular phosphorylation. The effects of insulin on adipose tissue, perhaps its major target organ, are fully discussed by Cahill and his colleagues. Five papers unveil the current basic disagreement concerning the ability of insulin to reduce hepatic glucose output.

The final section is more clinically orientated, and here the merits and demerits of the different sulfonylureas are amply delineated. Tolbutamide appears to be the safest. After the conference, metahexamide was withdrawn from experimental trial by the manufacturers on account of its toxicity. Chlorpropamide and the biguanides both have their champions, but their final place in therapy remains to be decided.

This monograph has been well and promptly produced, and lavishly illustrated.

DENIS ABELSON

THE STORY OF DISSECTION. By Jack Kevorkian. New York, Philosophical Library, Inc., 1959. ix, 80 pp. $3.75.

The author traces the story of human dissection from antiquity to the 20th century in a clear, concise manner. It is so concise, however, that unless the reader has a medical background he could neither fully appreciate nor adequately understand its contents. The philosophic concepts of the various historical ages which hindered or enhanced the performance of human dissection are very briefly but fairly adequately described. The author has neatly interwoven the history of the development of human dissection as an autopsy technique for the study of pathology and as a purely scientific discipline for the study of normal anatomy. However, human dissection in relationship to pathology receives much more emphasis than its relationship to anatomy as a scientific discipline. Therefore, the author does not relate how anatomists for a long period of time had to cooperate with often unscrupulous grave robbers in order to acquire cadavers for teaching and research purposes, frequently at the risk of endangering their lives, because of stong public sentiment and inadequate anatomical laws.

The principal value of the book is in its emphasis on the fact that the results of human cadaver dissections and necropsies performed over the ages comprise such a fundamental basis of modern medical practice and that during periods of history when dissections and necropsies were allowed, the field of medicine took great strides forward. The author points out that, "Without human dissection the superstructure of cellular pathology, physiopathology, and experimental pathology (and surgical pathology and all of surgery) would crumble into a heap of frippery which would be meaningless in a long range sense. Virchow himself emphasized the importance of conceiving the organism as a whole, a unity of structure and function, rather than as a mechanistic composite of autonomous parts, be they organs or cells." I concur with this viewpoint because dissection of the human cadaver not only allows a medical student to learn the various structures which comprise the body but how the structures are related to each other and to the whole body as a structural unit. Thus, the book has a timely message when the value of cadaver dissection by medical students as a basic requirement is now being adversely questioned. Unfortunately, the dissemination of the book's message may be greatly hindered by its price in relationship to its size because it contains only 80 pages (5¼ x 8¼ inches) of large type with sizeable margins.

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